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PREFACE

In offering this, our sixth volume of lectures, we do so with a confidence born of the encouragement and recognition the earlier series have won. Each year the function of the lectures in diffusing knowledge of the medical sciences seems more assured and we owe a constantly increasing debt to the authors of the lectures, for their generous services to the public.

To the editors of the *Journal of the American Medical Association, The American Journal of the Medical Sciences,* and *The Archives of Internal Medicine* we here acknowledge our obligation, for their consent to our republishing the lectures of Professors Chiari, Cushny, and Wells. The other lectures have not been published elsewhere.

On April 1, 1911, which was the three hundred and thirty-third anniversary of the birth of William Harvey, the occasion was commemorated by an address by Dr. S. Weir Mitchell, of Philadelphia, on William Harvey. This address being of especial interest from the literary and historical more than from the scientific point of view, and being less complete as a study of the great physiologist than the author expects ultimately to make it, has not been included in this volume.

Haven Emerson, *Secretary,*
120 East 62d Street, New York.

October, 1911

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THE IMPORTANCE OF THE AUTOPSY AND OTHER PATHOLOGIC-ANATOMIC EXAMINATIONS[1]

PROF. HANS CHIARI
University of Strasburg

IN 1761 Giovanni Battista Morgagni (1682–1771, Professor of Anatomy in Padua) published his famous work: "De Sedibus et Causis Morborum per Anatomen Indagatis," and thereby became the real founder of human pathology. He attempted to give a very complete picture of morbid processes by carefully comparing the clinical aspects of disease with the anatomic findings in a large number of cases. In the title of his work Morgagni summed up the chief problems of pathologic anatomy and in recognition of this fact Rokitansky (1844–1875 Professor of Pathology in Vienna) used the title in the following somewhat modified form, for the inscription on the Pathologic-Anatomic Institute in Vienna: "Sedibus et Causis Morborum per Anatomen Indagandis."

As the sphere of pathologic anatomy has broadened since it became an independent science seeking to solve its own problems, not only by the examination of the cadaver, but also in other ways—for instance, by the experimental method—the position of the pathologist has correspondingly increased in importance. In the autopsy room the pathologist must take due cognizance of the ever-broadening and more exacting demands of the clinician; he must endeavor at each autopsy to give the physician a satisfactory explanation, and he must examine each case most minutely with the aid of modern methods of histology and bacteriology. By means of such elucidation, he will materially assist the clinician, and he will also develop new

[1] Delivered October 15, 1910.

ideas in his own scientific field. In this way he can advance toward the goal of all the medical sciences, namely, the relief of human suffering.

Therefore there can be no doubt of the need of pathologic investigation. All the medical schools and hospitals are wise in striving for a large amount of autopsy material in order that the students may be instructed, that physicians may gain experience, and that the interests of science may be advanced. Naturally the autopsies must all be done with the strictest adherence to the principles of humanity, with careful consideration for the wishes of the relatives, and with the greatest precautions to avoid any mutilation of the body. To this end pathology has at its disposal a well-developed technic which is constantly being improved.

In order to give a graphic picture of the importance of postmortem examinations, I should like to select a series of cases from the eighty-seven autopsies performed at the Pathological Institute in Strasburg during the month of June, 1910, and to discuss them somewhat in detail:

ILLUSTRATIVE CASES: CLINICAL AND PATHOLOGIC-ANATOMIC DIAGNOSIS

Eleven cases of fatal nutritional disturbances in infants between the ages of 2 and 6 months: six cases of enteritis; five cases of general atrophy.

Two cases of status lymphaticus:

Patient, male, aged 16. Otitis media with meningitis and chronic general tuberculosis. Death while under chloroform narcosis at the beginning of the operation.

Patient, female, aged 27. Ruptured tubal pregnancy in second month. Salpingectomy seven hours after rupture. Death three and one-half hours later. Moderate anæmia.

Four cases of fatal embolism of the pulmonary artery:

Patient, female, aged 38. Death from thrombosis of the right femoral vein, four days after salpingectomy for gonorrhœal salpingitis.

Patient, female, aged 53. Death from thrombosis of the ovarian vein of the left side, six days after double ovariotomy for carcinoma.

Patient, female, aged 58. Death from thrombosis of the left external saphenous vein, six days after operation for femoral hernia.

Patient, female, aged 63. Death from thrombosis of the right external saphenous vein, twenty-two days after encephalomalacia.

Two cases of auto-intoxication from incarcerated hernia:

Patient, male, aged 73. Incarcerated inguinal hernia for five days. Death one day after operation for hernia. Epithelial necrosis of the kidneys.

Patient, female, aged 58. Incarcerated femoral hernia for four days. Death three days after operation for hernia.

Two cases of latent septicæmia:

Patient, female, aged 40. Clinical diagnosis: ileus from carcinoma of the sigmoid flexure. Pathologic-anatomic diagnosis: streptomycotic peritonitis caused by suppurative salpingitis.

Patient, female, aged 56. Clinical diagnosis: chloroform narcosis after hysterectomy for ulcerating carcinoma of the cervix. Death one day later. Pathologic-anatomic diagnosis: streptomycotic septicæmia.

Two cases of birth injuries:

Fœtus of 9 months. Breach extraction. Compression of skull and rupture of the spinal column between the fifth and sixth dorsal vertebræ.

Patient, female, aged 25. Transverse position. Laceration of the vagina following attempted version outside of the hospital.

One case of criminal abortion:

Patient, female, aged 24. Pyæmia after abortion. Laceration of the vagina, of the posterior labium, of the external os and of the cervix.

Patient, female, aged 38. Peritonitis from perforation of the rectum.

Two cases of latent carcinoma of the stomach:

Patient, male, aged 43. Clinical diagnosis: carcinoma of the sigmoid flexure. Pathologic-anatomic diagnosis: carcinoma of the pylorus and secondary carcinoma of the peritoneum, with stricture of the sigmoid and of the descending colon.

Patient, female, aged 27. Clinical diagnosis: carcinoma of the ovaries. Pathologic-anatomic diagnosis: carcinoma of the posterior gastric wall and secondary carcinoma of the ovaries.

One case of latent pericarditis:

Patient, female, aged 71. Clinical diagnosis: lobular pneumonia. Pathologic-anatomic diagnosis: lobular pneumonia, tuberculous pericarditis with effusion, latent tuberculosis of the apex of the lung.

One case of late recurrence of carcinoma:

Patient, male, aged 47. Recurrence of carcinoma in the cicatrix four years after excision of the carcinomatous rectum.

One case of puerperal eclampsia:

Patient, female, aged 37. Multiple necrosis of the liver.

One case of Hodgkin's disease:

Patient, male, aged 27. Swelling of the left lymphatic glands and left tonsil. Nodes in the spleen, lungs and pleuræ. (The same diagnosis was made nine months previously from an extirpated axillary gland.)

One case of nephrolithiasis:

Patient, female, aged 67. Right hydronephrosis and uratic calculi. Left uratic nephrolithiasis. (Calculi in left kidney stained with methylene blue.)

COMMENT

The cases of nutritional disturbances in infants confirm the frequently expressed opinion that serious digestive disturbances occur without even microscopic changes in the intestines, which depend entirely on chemical alterations in the intestinal contents. These chemical changes are being carefully studied of late by pediatrists and thereby a real basis for a correct therapy is being established.

The cases of status lymphaticus show the great significance of this constitutional anomaly. Patients of this class stand chloroform anæsthesia and loss of blood very poorly.

The four cases of fatal embolism of the pulmonary artery are good illustrations of the frequency of this lesion, which so often interferes with the success of operations. Such an embolism is not a rare occurrence, and it is easy to realize that surgeons take every precaution to avoid such a complication. This condition cannot always be diagnosed clinically with certainty, and therefore the autopsy is necessary to confirm the diagnosis.

The cases of incarcerated hernia serve to show how much danger attends the retention of the intestinal contents. Very commonly this decomposition of the intestinal contents causes an auto-intoxication to which the patients succumb in spite of a successfully performed herniotomy. In order to prove this point of view, I have but to refer to the first of the two cases in which the microscope showed diffuse necrosis of the renal epithelium. In other cases, on the contrary, the necrosis of the liver parenchyma is of special interest.

The two cases of septicæmia emphasize the importance of bacteriologic examinations at the post-mortem table. In the first case, no positive clinical diagnosis could be made. The autopsy showed a streptococcic peritonitis due to a purulent streptococcic salpingitis, which in turn, judging from the nature of the bacteria, was of puerperal origin. In the second case, streptococci were cultivated from the heart's blood and from the various tissues of the body, and the case therefore had to be diagnosed as a streptococcus septicæmia. Later, on talking the matter over, the clinician thought, and rightly, I believe, that the infection was due to the deep ulceration of the carcinoma of the cervix, which had been curetted.

The cases of birth injuries are of great importance because they can easily result in severe injury to mother and child. In the first case, the delivery was certainly difficult, and in spite of the fact that it was performed by a very competent obstetrician, it resulted in a fracture of the spinal column. In the second case, a physician not in the hospital had made several attempts to correct a complex presentation by means of version and had thereby lacerated the vagina so severely that there remained only a strip of its anterior wall behind the urethra. The patient was subsequently brought to the hospital and died of acute anæmia.

The case of criminal abortion shows the typical lesions which we find in this class of cases. Frequently there occur punctured wounds of the posterior wall of the vagina, perforations of the cul-de-sac of Douglas, injuries of the posterior lip of the cervix and of the adjoining portion of the uterus. As these injuries are for the most part done with unclean instruments, the women so treated are very often victims of fatal puerperal infections.

The case of injury due to enema, which happened to a 38-year-old woman outside of the hospital, may serve as a warning. Pathologic anatomy teaches that such injuries from enemas are due to the use of hard cannulas, and are, especially in children, much more frequent than is generally believed.

The cases of latent carcinoma of the stomach are very important from a diagnostic point of view. In the first case, the

secondary carcinoma of the peritoneum, which had invaded the sigmoid flexure and caused a stricture, was diagnosed as a primary lesion, and in the second case we were dealing with a condition frequently described of late, a primary carcinoma of the stomach with typical secondary implantation lesions in both ovaries in which the secondary metastases had been regarded as primary lesions.

In the same way, the case of latent pericarditis demonstrates the difficulty of many a clinical diagnosis.

The case of recurrent carcinoma in the scar four years after extirpation of the rectum for carcinoma, is instructive because this recurrence was a very late one. We now have, however, definite knowledge of much later relapses.

The case of puerperal eclampsia was typical. It showed perfectly the characteristic areas of necrosis and hemorrhage of the liver, which were first described by Schmorl.

In the case of Hodgkin's disease there was found the well-known histologic condition of granulation tissue with fibrous connective tissue and polynuclear giant-cells in all the diseased areas; these lesions had also been found in the axillary glands, which had previously been extirpated for diagnostic purposes.

The last case had the significance of an experiment. The 67-year-old woman had been given methylene blue on account of symptoms of cystitis. At the autopsy, the uratic calculi in the left kidney, which secreted urine, were stained with methylene blue, whereas those in the right kidney, which had ceased to functionate as a result of atrophy following hydronephrosis, were entirely free from any stain.

These cases, selected from the records of one month, well demonstrate the importance of the careful routine autopsy and indicate how much the student and physician can learn both from a scientific aspect and from the point of view of the welfare of the patient. Every autopsy adds to our store of knowledge and justifies the old adage which we often see inscribed in the autopsy room: "Hic est locus ubi mors gaudet succurrere vitæ."

A pathologic institute can be of service to the physician and

of benefit to the sick in other ways. I refer to the microscopic diagnosis of tissue removed by the surgeon. I may here mention the results of the microscopic examination of curettings from the uterus. How often the examination does not determine whether we are dealing with an abortion, an endometritis or a carcinoma!

Furthermore, I may refer to the microscopic examinations for carcinoma of excised tissue, a recourse which so often saves the patient from the sacrifice of important organs, *e.g.*, the tongue, the larynx, the penis, the cervix. On the other hand, if the carcinoma or other neoplasm is diagnosed, it may lead to an immediate life-saving operation. Finally, I wish to emphasize the importance of a careful examination of excised tumors, for this not infrequently, to the gratification of the pathologist, demonstrates that a condition which has been considered malign is in reality benign and permits of a favorable prognosis. These investigations are indeed very difficult and the pathologist cannot be too careful in his reports; otherwise there is danger that he will bring undeserved discredit on the value of diagnoses made from excised tissue. He must never hesitate to acknowledge that he is unable to render a definite diagnosis. Then he will rightly fulfil his duty and the clinician will appreciate the weight of his opinions.

Thus it is seen how important is the function of the pathologist in performing autopsies, and how his calling is not only didactic but humanitarian. His work, therefore, deserves support and encouragement from all sides, and especially from the laity. The public must be made to appreciate to an ever-increasing extent the importance of pathologic anatomy. That this hope may soon be realized is my earnest wish, especially for the American universities.

HOW DOES THE ACT OF FERTILIZATION SAVE THE LIFE OF THE EGG?[1]

PROF. JACQUES LOEB
The Rockefeller Institute for Medical Research

THE unfertilized egg dies in a comparatively short time, while the act of fertilization gives rise to a series of generations which, theoretically at least, is of infinite duration. The act of fertilization is, therefore, a life-saving act for the egg. The problem arises, in which way can the spermatozoön save the egg's life?

If the ovaries of a star-fish are put into sea-water the eggs are shed. They are generally immature, and in this condition they cannot be fertilized, either by spermatozoön or by chemical means. If they remain, however, for some time in sea-water, all or a number of them gradually become mature; that is to say, their nuclear mass is diminished by the extrusion of two so-called polar bodies. If immediately after the extrusion of the polar bodies sperm is added, the eggs develop. They can at that period likewise be caused to develop by certain chemical and physical agencies.

Ten years ago I made the following observations: If the eggs are not caused to develop by sperm or by physiochemical agencies, they perish very rapidly. At summer temperature they may die in from four to six hours. The death of the egg manifests itself morphologically in a darkening and blackening of the otherwise clear egg. I found that the death of the egg can be prevented by withdrawing the oxygen, or by diminishing the rate of oxidations in the egg through the addition of a trace of potassium cyanide. The life-saving action of lack of oxygen can be shown in various ways. The maturation of the egg itself depends upon oxidations. If one takes away the oxygen from the immature eggs, or if the oxidations in the immature eggs are inhibited by potassium cyanide, the process

[1] Delivered November 19, 1910.

of maturation does not take place. Maturation is, therefore, also a function of oxidations. The eggs of a female, which were unripe, were divided into two groups: the one group remained in sea-water in contact with oxygen; the other was put into sea-water whose oxygen had been removed by a current of hydrogen. The eggs of the second group remained alive; the eggs of the first group perished in a few hours.

It is not even necessary to drive out the air by hydrogen; one can preserve the life of the unfertilized eggs also by putting large masses of them into a narrow glass tube which is sealed at the bottom. The eggs sink down to the bottom of the tube, and those which are lying near the bottom receive no oxygen, since the oxygen which diffuses from the air through the sea-water is consumed by the uppermost layer of the eggs. On account of this lack of oxygen the eggs lying at the bottom of the tube do not mature and do not perish; hence by withholding oxygen from the immature eggs their maturation and their death are prevented. This might perhaps not be so strange, but the following result is much more strange.

If the oxygen is withheld from the eggs immediately after they become mature their life is also saved. A. P. Mathews has repeated this experiment and obtained the same results. This proves that the death of the mature but unfertilized egg is determined by oxidations. If these oxidations are inhibited death does not occur. When these experiments were published they first caused opposition. This opposition was founded on the application of potassium cyanide in part of the experiments. The objection was raised that the potassium cyanide in these experiments only acted by preventing the development of bacteria. The authors, however, who raised this objection, overlooked the fact that lack of oxygen acts in exactly the same way as the addition of potassium cyanide, and that it is entirely immaterial how lack of oxygen is produced, whether the oxygen is driven out by carefully purified hydrogen or whether the eggs are put together into a large heap, whereby only those lying on the surface of the heap receive enough oxygen.

It is, however, easy to show directly that the above-mentioned objection is incorrect. It is not difficult to put the eggs of the star-fish, without bacterial infection, into sterilized sea-water. The following experiment was tried. The eggs of a star-fish were separated into three parts: one part was put aseptically into a series of flasks with sterilized sea-water; the second part was put into ordinary sea-water without asepsis; the third part was put into sea-water to which a large quantity of a putrid culture of bacteria, that had developed on the dead eggs of the star-fish, had been added. It was found that in all three cases the mature eggs died within the same time. The sterilization of the eggs of the first group was complete, as was shown by the fact that the eggs kept in flasks for two months preserved their form, while the dead eggs in the normal sea-water were destroyed in a few days by the action of the bacteria.

It is, therefore, certain that the death of the star-fish eggs which are not fertilized is not caused by bacteria, but by the process of oxidations in the egg. If no spermatozoön enters the egg, or if the egg is not caused to develop in a chemical way, it perishes very rapidly. If, however, a spermatozoön enters the egg, it remains alive in spite of the fact that the entrance of the spermatozoön causes a relative acceleration of the oxidations in the egg. Warburg found for the eggs of the sea-urchin at Naples that fertilization raises the velocity of the process of oxidations to six times their original value, while Wasteneys and I found that fertilization caused an increase in the velocity of oxidations of Arbacia in Woods Hole to three or four times the rate found in the unfertilized eggs.

How can we explain that fertilization saves the life of the egg? Let us make the following preliminary assumption: The unfertilized egg contains a poison, or some faulty combination of conditions which, if oxidations take place, cause the death of the egg. In the unfertilized but mature egg oxidations take place. The spermatozoön carries among others a substance into the egg which protects the egg against the fatal effects of the oxidations, and allows them even to carry on

oxidations at an increased rate without suffering. We might say that the mature but unfertilized egg is comparable to an anaërobic being for which oxidations are fatal, and that the spermatozoön transforms the egg into an aërobic organism.

If we compare the eggs of different animals, we find great differences in regard to the above-mentioned conditions. The eggs of certain annelids (Polynoe) also perish rapidly if they become mature without being caused to develop, while the eggs of the sea-urchin remain alive for a longer period of time after they have become mature. It has not yet been investigated what determines this difference.

II

The analysis of the process of fertilization by the spermatozoön shows that we must discriminate between two kinds of effects, the hereditary effect and the activating or developmental effect. The experiments on artificial parthenogenesis make it very probable that the two groups of substances: the substances which determine the heredity of paternal characters and the substances which cause the egg to develop, are entirely different. In this paper we are only concerned with the second group of substances, namely, those which cause the development of the egg.

The analysis of the causation of development of the egg by a spermatozoön has shown that the latter acts by carrying at least two substances or groups of substances into the egg. The one of these substances causes the formation of a membrane; the second serves the purpose of rendering the egg immune against the fatal action of oxidations.

I have shown in a number of papers that the essential feature in the causation of development of the egg is a modification of its surface, which in many cases leads to the formation of a membrane. If we cause membrane formation in an unfertilized sea-urchin egg by artificial means, it begins to develop, but it perishes very soon; much more rapidly than if it is not exposed to any treatment. I was able to show that this rapid death of the sea-urchin egg, after artificial membrane

formation, can be prevented either by withdrawing the oxygen from the egg or by inhibiting the oxidations in the egg through the addition of a trace of potassium cyanide. The membrane formation, therefore, causes the rapid death of the egg through an acceleration of oxidations. Warburg has recently shown that the artificial membrane formation in the unfertilized sea-urchin egg causes the same increase in the rapidity of oxidations as the entrance of a spermatozoön.

If we wish to cause the unfertilized eggs to develop to the pluteus stage after the membrane formation, we have to subject them to a second treatment. This may consist in putting them about 15 minutes after the membrane formation into a hypertonic solution of a certain osmotic pressure (for instance, 50 c.c. of sea-water + 8 c.c. N/2½ NaCl) for one-half to one hour. If, after this time, they are put back into normal sea-water they no longer perish, but develop into normal larvæ. I ventured the hypothesis that the artificial membrane formation causes a rapid increase of the oxidations in the egg and in this way causes it to develop, but that these oxidations lead to the rapid decay of the eggs at room temperature for the reason that the egg contains a toxic substance, or a toxic complex of conditions, which in the presence of oxidations leads to the rapid death of the egg. The second treatment serves the purpose of rendering the egg immune against the toxic effects of the oxidations.

If we first cause the artificial membrane formation in the unfertilized egg by any of the various means which I have described in former papers, and if we afterward treat the eggs for a short time with a hypertonic solution, they develop after being transferred to normal sea-water in the same way as if a spermatozoön had entered them. They reach the successive larval stages, develop into a blastula, gastrula and pluteus, and live as long as the larvæ produced from eggs fertilized by a spermatozoön.

Hence the physicochemical activation of the unfertilized egg of the sea-urchin consists of two kinds of treatment. The one is a change in the surface of the egg which may or may not result in the so-called formation of the membrane. This

change causes the acceleration of oxidations which in my opinion is the essential feature of the process of fertilization. The second treatment consists in abolishing the faulty condition which makes oxidations fatal to the egg. This second treatment may consist in exposing the eggs for about half an hour or a little more to a hypertonic solution. We can substitute, however, for this treatment another treatment, namely, the deprivation of the egg for three hours from oxidations, either by removing the oxygen from the solution or by adding a trace of potassium cyanide to the solution. If, after the treatment with the hypertonic solution for half an hour, or the treatment with lack of oxygen for about three hours, the eggs are put back into normal sea-water they can develop normally into normal larvæ.

We can show that the spermatozoön also causes the development of the egg by two different agencies comparable in their action to the agencies used in the methods of chemical fertilization which we have just described.

For this purpose we must fertilize the egg of the sea-urchin with a sperm different from its own, and for the following reason: The spermatozoön of the sea-urchin enters so rapidly into the egg of the sea-urchin that it is impossible to show that it causes the development of the egg by two different substances.

If, however, we fertilize the sea-urchin egg with the sperm of star-fish, it takes from ten to fifty minutes to cause the membrane formation in the eggs, the reason being that the star-fish sperm can penetrate only very slowly into the egg of the sea-urchin.

It is, as a rule, not possible to fertilize the egg of the sea-urchin by star-fish sperm in normal sea-water. But I found eight years ago that if we make the sea-water slightly more alkaline than it naturally is the eggs of the sea-urchin can be fertilized by the sperm of the star-fish. For the fertilization of the Californian sea-urchin, Strongylocentrotus purpuratus, with the sperm of Asterias, the best results were obtained when 0.6 c.c. of N/10 NaOH were added to 50 c.c. of sea-water. In

that case, with active sperm, in about fifty minutes all the eggs form the typical fertilization membrane.

If one watches the further development of sea-urchin eggs fertilized by star-fish sperm one notices very soon that there are two different kinds of eggs present; the one kind of eggs behave as if they had been fertilized with sperm of their own kind. That means, they segment regularly and develop into swimming blastulæ and gastrulæ. The other kind of eggs, however, act as if they had been treated with one of the agencies which cause the membrane formation in the unfertilized sea-urchin egg; these eggs begin to segment, but at room temperature they slowly perish by cytolysis. If, however, these eggs are treated for half an hour with a hypertonic solution they develop into larvæ.

If one examines the eggs of a sea-urchin which have been treated in an alkaline medium with the sperm of the star-fish, one finds that only a certain percentage of these eggs contain the sperm nucleus, and this percentage seems to be identical with the percentage of the eggs which develop into larvæ. As far as the other eggs are concerned, which only form a membrane and then disintegrate, no sperm nucleus can be found inside of them. I am inclined to draw the following conclusion from these observations: The spermatozoön of the star-fish penetrates very slowly through the surface film of the sea-urchin egg. When it lingers for some time partially imbedded in the surface film, one of the substances of the spermatozoön is dissolved in the superficial layer of the egg and causes the membrane formation. Through the act of membrane formation the further entrance of the spermatozoön into the egg is prevented; since the fertilization membrane is impermeable to sperm. This membrane formation leads to an increase in the rate of oxidations and the beginning of the development of the egg. The latter, however, contains a toxic substance, or a faulty complex of conditions which has to be abolished, before the oxidations necessary for development can take place without the egg being destroyed by them. The spermatozoön carries a second substance into the egg which renders it immune

against the fatal actions of the oxidations. While the membrane forming substance of the spermatozoön may be situated at its surface, or superficially at least, the second substance which transforms the egg from an anaërobe into an aërobe must be situated in the interior of the spermatozoön; since it can only act if the spermatozoön penetrates into the egg. We see in these observations, concerning the fertilization of the sea-urchin egg by the star-fish sperm, a proof that the activation of the egg by the spermatozoön is also caused by two different substances, one of which causes the membrane formation, while the second renders the egg immune against the toxic action of the oxidations. These data support the assumption made above that the life-saving action of the spermatozoön is due to the fact that it carries a substance into the egg which renders the latter immune against the toxic action of oxidations.

III

Seven years ago I found that a number of agencies destroy the fertilized egg much more rapidly than the unfertilized egg. Thus, for instance, while in a pure sodium chloride solution the unfertilized egg of the Californian sea-urchin may be kept alive for several days, the fertilized egg is destroyed in such a solution in less than twenty-four hours. If we use slightly alkaline solutions of sodium chloride the greater resistance of the unfertilized egg is perhaps still more striking. The egg of Arbacia is cytolyzed in a neutral sodium chloride solution in a few hours, while the unfertilized egg may live for a considerably longer period of time. When we put fertilized eggs and unfertilized eggs into hypertonic solutions, we find also that the fertilized eggs suffer much more than the unfertilized eggs. What causes this difference of sensitiveness between fertilized eggs and unfertilized eggs? It is possible that the permeability of the fertilized eggs is greater than that of the unfertilized eggs. While this is probably to some extent true, yet it is not the whole explanation of the difference in the behavior of the two kinds of eggs. I have been able to show for a number

of toxic solutions that their effect can be either completely annihilated or at least diminished if we take the oxygen away from the solution. Thus, for instance, fertilized eggs of the sea-urchin which perish very rapidly in pure salt solutions, or a solution of sodium + calcium, or a solution of sodium + barium, can be kept alive for a considerable period of time in the same solutions if we either carefully remove the oxygen from the solutions, or if we diminish the rate of the oxidations in the eggs by adding a trace of sodium cyanide. In this case we have the direct proof that solutions which are fatal for the egg when the oxidations are allowed to go on are rendered completely, or at least partially, harmless if we stop the oxidations in the egg. Not only the toxic action of salt solutions upon the fertilized egg could be inhibited by the suppression of the oxidations in the egg, but also the toxic action of sugar solutions, or of solutions of alcohol in the sea-water, or of a solution of chloral hydrate.

These observations prove directly that in the presence of certain toxic substances or mixtures of substances the oxidations in the egg lead to its rapid destruction; while a suppression of the oxidation saves the life of the egg.

We, therefore, believe that we may conclude that the rapid death of the unfertilized egg of certain species is caused by the oxidations which take place in these eggs; and that the life-saving action of the spermatozoön consists in the fact that the latter, in addition to the membrane-forming substance, carries a second substance, or group of substances, into the egg which renders the egg immune against the harmful effect or consequences of oxidations.

DYSPITUITARISM *

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OUR slowly acquired comprehension of the disorders of the thyroid and parathyroid glands resembles, in the deviousness of its progress, the steps which have been taken in the approach toward a better understanding of disorders of the pituitary body. In both instances surgery has played a not unimportant rôle, in laboratory as well as in clinic, more especially in throwing light on the consequences of diminished function due to grandular extirpations—a negative method, to be sure, of studying glandular activity.

Owing to the more obvious complicity of the thyroid in certain clinical syndromes, as well as its greater surgical accessibility, it was made the object of operative attack long before this was ventured in the case of the hypophysis; but in both instances, as may be recalled, it was the coincident enlargement or tumor of the gland which first suggested its active and possibly causal participation in the newly recognized constitutional maladies.

Had it not been for the neighborhood pressure symptoms, especially those of tracheal distortion, the early operations for goitre (1883-1886) by Kocher and Reverdin would not, in all probability, have been undertaken, and the experimental explanation of Gull's myxœdema (1873) might have been much longer delayed. Had it not been for the obvious tumor

* This lecture, of December 10, 1910, owing to its extensive case reports and copious illustrations, does not lend itself in its original forms to publication in this series. The author has incorporated much new material since the lecture was delivered, and the J. B. Lippincott Company will publish it in full as a separate monograph. The accompanying text is an excerpt and gives the clinical subdivisions in accordance with which the author has provisionally grouped his numerous illustrations of hypophyseal disease. *Editor.*

of the pituitary body, which likewise causes its own characteristic neighborhood symptoms, Marie and Marienesco (1891) probably would not have come to associate acromegaly with, and Fröhlich (1901) certainly would not have attributed adiposity and sexual infantilism to a lesion of this supposedly unimportant and vestigial structure.

In the investigations concerning the functions of the hypophysis, which must date from Marie's discovery, many lessons have been drawn from the earlier experiences with the thyroid, and, difficult as the problem is, many confusions have been avoided by bearing in mind the pit-falls into which the earlier thyroid investigations stumbled. This applies particularly to the fact that there was a long overlooked double glandular representation,—thyroid and parathyroid,—for advantage was not taken of Sandström's discovery until Gley's experimental demonstration of the functional significance of these lesser bodies led to the unravelling of many obscurities regarding supposed states of athyroidism as experimentally induced in animals. This should have warned us at the outset of the possible confusion incidental to the study of the function of the pituitary body as a whole; nevertheless, even now insufficient attention is paid to the two chief subdivisions of the gland, which have such diverse physiological properties.

Now, in the case of thyroid gland, separate from its accessory glandules, a fairly satisfactory working basis was established through a combination of laboratory and clinical experiences, with a symptomatology attributed to conditions of overaction of the gland—*hyperthyroidism,* with Graves's disease as its clinical prototype—and another due to glandular loss or inactivity—*hypothyroidism,* with Gull's myxœdema as its typical adult and cretinism as its typical childhood manifestation.

This basis proved satisfactory enough for a time, and extreme grades of these opposed states are now unmistakable. However, further experience has shown that the symptomatology of the two types may merge to a surprising degree,

certain clinical manifestations of exophthalmic goitre often being overlapped by those supposedly characterizing myxœdema. Surgical misjudgments, more than any other factor, have made this clear; many patients, for the simple reason that nervousness, tachycardia, and exophthalmos persisted or returned after earlier operations, having been again subjected to arterial ligation or partial thyroidectomy, despite the fact that a dry and puffy skin, a subnormal temperature and what not indicated the early signs of glandular insufficiency. Indeed, as Marine has so clearly pointed out, oft-repeated stages of glandular overactivity tend toward a final stage of pathological glandular sluggishness, and the transition symptoms may be neither one thing nor the other, neither hyper- nor hypothyroidism, but rather *dysthyroidism*. Now, these same principles, as we shall see, are equally applicable to disorders of the pituitary gland.

In an earlier paper,[1] the contents of which were founded more upon experimental than upon clinical experiences, the two usual subdivisions, which had long served a useful purpose in the case of the thyroid, were taken as a temporary working basis: namely, states of overactivity (*hyperpituitarism*) and states of underactivity (*hypopituitarism*). These simple subdivisions, however, often prove clinically misleading, particularly in the presence of such syndromes as have at the outset been called into being by presumed conditions of primary overactivity. For these become blended later on with symptoms equally characteristic of functional insufficiency, so that although—disregarding for the moment the separate lobes—states of hyper- and of hypopituitarism are distinguishable, evidences of one state are not uncommonly superimposed on those of the other, and it may be difficult to tell which predominates. Hence the term *dyspituitarism* becomes in the majority of cases more appropriate.

But even accepting dyspituitarism as a sufficient mantle for

[1] The hypophysis cerebri, Journ. of the Am. Med. Assn., July 24, 1909.

all instances of perverted function of the hypophysis, we nevertheless are still so far from complete knowledge of the various clinical syndromes which may be elicited by disturbances primary in one or the other lobe, that, for a time at least, we shall do well to adhere to the more cumbersome though temporarily useful clinical subdivisions. For if not carried to the point of confusion through excessive subdivisions, the separation, on the basis of certain striking clinical features, of the various types of disease attributable to lesions of a given organ may serve as a temporary convenience, even though such symptomatic pigeon-holing does not prove to be of enduring value. This, of course, harks back to Morgagni's " De sedibus et causis morborum." Unquestionably the day is rapidly dawning which will disclose the chemical or other influences at work behind the gross alterations in a given organ long styled " the seat of disease " by the morbid anatomist.

In an analysis of our thirty or forty cases of obvious hypophyseal implication, a tentative grouping was first made into, (1) those in which the gland was seemingly the primary seat of disease, and (2) those in which it was secondarily involved, usually by the direct compression of an adjoining, or the more remote effects of a distant, cerebral lesion. However, on clinical grounds, it often proved impossible to tell, in the first place, whether or not many of the interpeduncular tumors were actually glandular in origin; and secondly, recognizable hypophyseal symptoms brought about by distant lesions—cerebral tumors for example—proved to be so uniformly present that this simple subdivision was abandoned in favor of one comprising a larger number of clinical groups—six, as a matter of fact. These provisional groups are as follows:

I. *Cases of dyspituitarism in which not only the signs indicating distortion of neighboring structures but also the constitutional effects of altered glandular activity are outspoken.*

Here we have on the one hand the so-called neighborhood signs and symptoms tell-tale of a growth in the interpeduncular region, and it matters little whether the lesion has originated

in an intra- or extrasellar anlage. Of primary diagnostic importance are the changes in the configuration of the pituitary fossa which the X-ray betray. Secondly come the signs of pressure implication of the adjoining cerebral nerves, the most characteristic being the primary optic atrophy with accompanying constrictions of the field of vision, a bitemporal defect being the most striking though far from the most frequent type of perimetric distortion. Oculomotor palsies are common, and involvement of olfactorius and trigeminus may occur. The cerebrum itself may become distorted, and spasticity may indicate pressure involvement of the crura; gustatory and olfactory fits may indicate involvement of the uncinate gyri; psychic disturbances, involvement of the subjacent frontal lobes. Thirdly, there may be signs referable to the nasopharynx. Epistaxis is common. An intermittent discharge of mucus from the sphenoidal cells may occur and be misinterpreted as sphenoidal sinus disease. Occasionally there is a true cerebrospinal rhinorrhœa. The tumor may actually project into the upper pharynx so as to be visible, or an enlarged accessory hypophysis may be detected.

On the other hand, the constitutional effects of the disturbed functional activity of the gland may vary from those elicited by a condition of primary hyperactivity to those characterizing primary glandular underactivity, and finally, and what is more common, there may be evidences of a commingling of these two states.

Most of the growths which arise from an infundibular anlage and secondarily implicate the hypophysis fall in this group, though it also comprises many of the primary glandular strumas which have burst through the pituitary capsule and invaded the interpeduncular region.

II. *Cases in which the neighborhood manifestations are pronounced but the constitutional symptoms inconspicuous.*

Here the same regional signs of tumor that have been recounted above are in evidence, but the disturbances of hypophyseal activity are so slight that they would unquestionably

be overlooked on a cursory examination. Close scrutiny, however, will often disclose some traces of skeletal change, either on the side of over- or undergrowth, and some evidences of disturbed carbohydrate metabolism, with a tendency to adiposity.

In this group fall most of the cases, with primary hypophyseal struma of chromophobe cell type, which have not advanced to the stage of hypopituitarism with adiposity and its allied symptoms as dominant features of the clinical picture. These are the cases which promise most from surgical therapy.

III. *Cases in which neighborhood manifestations are absent or inconspicuous, though glandular symptoms are pronounced and unmistakable.*

Here we meet with the constitutional evidences of altered glandular activity unobscured by actual tumor manifestations. For though an enlargement of the sella is usually to be made out in the individuals of this group with hyperpituitarism, the glandular hypertrophy or hyperplasia is usually insufficient to cause, at all events permanent, damage of adjoining structures.

Among the more important indications of altered glandular function are skeletal changes, whether on the side of overgrowth or undergrowth—a pars anterior affair, as we believe. Disturbances of carbohydrate metabolism, on the other hand, are chiefly a matter of modified posterior lobe activity, whether occurring as a lowering of the assimilation limit, which is commonly associated with the early stages of hyperpituitarism (acromegaly) or as a great increase in tolerance, such as characterizes all states of hypopituitarism. Coupled with the extraordinarily high tolerance for sugars in advanced stages of posterior lobe insufficiency, there is a tendency toward the deposition of fat (hypophyseal adiposity, or adiposis universalis), with a subnormal temperature, slowed pulse, a dry skin, loss of hair, and so on—symptoms, many of them indicative merely of a lowered oxidizing capacity of the organism, which can be largely set aside by organotherapy.

Most of the acromegalics of our series, and three of the cases of gigantism, fall in this group; and, what is to be emphasized, all but two of these individuals with the skeletal evidences of past hyperpituitarism at present exhibit unmistakable signs of existent glandular insufficiency or dyspituitarism.

The cases, on the other hand, in which hypopituitarism has been the primary factor are a little more difficult to certify unless they originate in childhood and are associated with skeletal undergrowth, incomplete sexual adolescence, and changes in the other ductless glands. In the adult, adiposity, high sugar tolerance, subnormal temperature, psychic manifestations, sexual infantilism of the reversive type and so on, represent such a common clinical picture that it is often venturesome, in spite of the experimental counterpart of this syndrome which is produced by hypophysectomy, to attribute the condition to hypopituitarism without the co-existence of hypophyseal neighborhood symptoms—which merely goes to show how dependent we have been in the past on the presence of a tumor in arriving at a diagnosis.

IV. *Cases in which obvious distant cerebral lesions are accompanied by symptomatic indications of secondary pituitary involvement.*

Not until it was shown with Goetsch[2] that the posterior lobe discharges its secretion into the cerebrospinal fluid, and with Goetsch and Jacobson[3] that the insufficiency of posterior lobe secretion is chiefly responsible for the high sugar tolerance and presumably for the adiposity, low temperature, and other symptoms in states of hypopituitarism, did it become apparent that hypophyseal manifestations were being shown by many individuals in whom an internal hydrocephalus had been caused by lesions—tumors or otherwise—distant from the sellar region.

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[2] Concerning the secretion of the infundibular lobe of the pituitary body and its presence in the cerebrospinal fluid. Am. J. Physiol., 1910, xxvii, p. 60.

[3] Carbohydrate tolerance and the posterior lobe of the hypophysis cerebri. Johns Hopkins Hosp. Bull., 1911, xxii, p. 165.

It has long been appreciated that the nutrition of patients afflicted with an obstructive hydrops, from one cause or another, is often retained to a surprising degree, even though they do not actually become obese; but without the knowledge of the active participation in metabolic processes played by the posterior lobe secretion, and of the fact that it enters the blood stream by way of the intraventricular cerebrospinal fluid, it would hardly have been conjectured that the constitutional symptoms which these patients were showing were attributable to an hypophyseal disorder.

Any lesion, whether inflammatory, neoplastic, or otherwise, which causes an hydrops of the third ventricle will elicit these symptoms in certain measure. So common, indeed, are they that it has become a routine with us in every complete neurological examination of patients with intracranial lesions to tabulate the possible hypophyseal symptoms with those referable to the nervous system itself.

Naturally the constitutional manifestations of obstructive dyspituitarism of this type will usually be nothing more than the symptomatic expression of diminished posterior lobe secretion, resembling in its clinical aspects the posterior lobe deficiency brought about either by a posterior lobe involvement in a destructive intrasellar lesion or by the obstructive effects of a superimposed interpeduncular growth. In our series of tumor cases, however, there occurs a striking example of acute hyperpituitarism (acromegaly) in association with a cerebellar cyst causing internal hydrocephalus. Hence it is not impossible that under certain conditions a coincident pars anterior hyperplasia may be called into being by the same influence which blocks the outlet of the posterior lobe secretion.

V. *Cases with a polyglandular syndrome in which the functional disturbances on the part of the hypophysis are merely one, and not a predominating, feature of a general involvement of the ductless glands.*

The close functional interrelation of all of the glands of internal secretion has become a matter of common knowledge. Presumably no lesion of any member of the series can occur without some physiological readjustment in the activity of all

the others. In a sense, therefore, a morbid process, whether productive of a condition of over- or underactivity of any individual member of the series, is, from the outset, a polyglandular affair, and leads in time to secondary symptoms on the part of the other glands, many of which we are learning to recognize and to interpret.

We are more familiar with the secondary changes resultant upon destructive lesions, whether experimentally produced or the result of disease, than we are with the readjustments due to morbid hyperplasias. However, a polyglandular syndrome will doubtless come to be recognized for both of these states and for every gland.

Owing to the difficulty of reproducing them experimentally, the functional hyperplasias—barring hyperthyroidism, hyperpituitarism, and possibly hyperthymism—are little understood, and in the case of the best known—hyperthyroidism—clinicians have paid little attention to its polyglandular manifestations. Doubtless the pineal, parathyroid, and adrenal glands, and the organs of Leydig and Langerhans as well, exhibit a definite clinical syndrome expressive of a state of overactivity, which awaits an Addison or a Gull, a Marie or a Graves to interpret for us.

We already have some clinical knowledge of the constitutional states due to functional insufficiency of interstitial cells of testis and ovary, of parathyroid and thyroid, of pancreatic islets, and adrenal, and, newly, of thymus, pineal, and hypophysis. For each of these states the syndrome is doubtless a polyglandular affair, though in not all is the interrelation so apparent as in the case of the pituitary gland, which has a particularly close physiological interaction with the reproductive glands. Nevertheless, despite the secondary polyglandular consequences of a process originating in any one of the glands, there is unquestionably for each of them a definite clinical syndrome which ordinarily is sufficiently expressive to indicate which member of the series is primarily affected by a morbid state of over- or underactivity.

As yet, however, there occur certain polyglandular syn-

dromes which, with our present insufficient knowledge, it is difficult to attribute with definiteness to a lesion primarily of one or another member of the series, and, as in a number of individual cases exhibiting a confused syndrome of this kind there exist unmistakable hypophyseal manifestations, this special group has been made to include such of them as have shown improvement under hypophyseal organotherapy.

VI. *The transient hypophyseal symptoms demonstrable in various states.*

In this group are included, in the first place, such functional alterations as occur during most of the trying epochs of life—in adolescence, pregnancy, and the climacteric, for example—when these alterations deviate, as is not uncommon, from what may be considered the physiological normal.

The hypophyseal disturbances which occur at times in *pregnancy* may be mentioned in illustration. It has long been known that the gland enlarges during the gravid state; and Erdheim has shown that a characteristic histological change takes place in the anterior lobe—a chromophobe hyperplasia. Not only may the enlargement of the gland be sufficient to cause hypophyseal headaches from distention of its dural envelope, and occasionally a transient bitemporal hemianopsia from pressure on the chiasm, but what is more remarkable, transient constitutional evidences of functional hyperplasia, as an enlargement of the sacral soft parts, hands and feet, lips, nose and face, may occur in company with glycosuria and other signs of transient hyperpituitarism. These hypophyseal manifestations are of course merely one feature of the polyglandular functional readjustment which occurs in pregnancy, the occasional outcrop of signs of hyperthyroidism being better known. In either case the disturbance is apt to be but temporary, and only when exaggerated can it be regarded as more than the normal physiological interreaction to the primary ovarian (luteal?) change.

In the second place, this group may be made to include those individuals in whom more or less obvious hypophyseal manifestations have occurred as the result of a *cranial injury,*

in the course of certain *infectious maladies,* or in association with cerebral *vascular disease* which has in one way or another (by aneurismal formation or by obliterative endarteritis) affected the blood supply to the gland. Finally, and of great importance, is the relationship between certain types of epilepsy and states of hypopituitarism.

Under the first four of these groups there will naturally occur three subdivisions, namely: (1) the cases in which the clinical manifestations of past or existing *hyperpituitarism* predominate (more particularly overgrowth, resulting in gigantism when the process antedates ossification of the epiphyses—*Typus Launois;* and in acromegaly when it is of later occurrence—*Typus Marie*) ; (2) those in which the clinical manifestations of *hypopituitarism* predominate (adiposity, with a persistence of both skeletal and sexual infantilism when the process originates in childhood—*Typus Fröhlich*; and with sexual infantilism of the reversive form when it originates in the adult—the type we have explained on experimental grounds and of which clinical illustrations are to be given); and (3) the mixed or *transition cases* exhibiting some features of both states—in other words, with evident *dyspituitarism.*

It will be possible to give examples, from our series, of the majority of these types, and though gaps will occur, these can be easily supplied from the great number of individual case reports which crowd the literature on the subject.

The subdivision of cases in the first three main groups according to the presence or absence of neighborhood disturbances, attributable to the local pressure effects of a tumor or glandular enlargement, seems advisable, not only in view of the important rôle which from the first an existing tumor has played in calling attention to the actual seat of disease, but also for the reason that we must still depend largely on its presence in many cases to certify the existence of hypophyseal implication, whether the gland be actually the primary seat of the growth or whether its disturbed function is merely a secondary consequence of a neighboring lesion.

That there are obvious crudities in this provisional method

of grouping these patients is freely acknowledged. One striking fault lies in the fact that it does not take into consideration the anatomical subdivisions of the gland, for we may doubtless have either an over- or underactivity of both the anterior and the posterior lobe or of either one alone. The pars anterior, so far as we can tell, not only seems to be more closely correlated with the other ductless glands, but presides more intimately over skeletal growth; whereas the posterior lobe has been shown to be more closely allied to the processes of tissue metabolism and to the activity of the renal and vascular systems.

Hence there occur in our series individuals whose overgrowth suggests a former state of glandular overactivity of one or possibly of both lobes, but who in time have acquired evidences of insufficiency of both lobes or of the posterior lobe alone. Other individuals suggest, by their undergrowth and adiposity as well as by their high sugar tolerance, that there has been an early functional interference with both lobes. Still others, from the outset, have shown evidences of posterior lobe deficiency alone and one individual shows a composite of anterior lobe hyperplasia and the coincident obstructive effects of posterior lobe secretion due to the distant lesion in the cerebellum. So instances might be multiplied.

We therefore may have combinations of inactivity of the posterior lobe with overactivity of the anterior lobe; a combination of overactivity of the posterior lobe with anterior lobe deficiency; and finally a combination showing either overactivity of both lobes or deficiency of both lobes. All of these possible states, furthermore, may occur either before or after adolescence, leading, as might be conjectured, to very different clinical pictures, which in time may come to be sufficiently well unravelled to justify a classification on a new basis. But in the present state of our knowledge of the different rôles played by the two lobes in disease it would be unsafe in many instances to draw conclusions as to their individual participation in the given syndrome.

Without the co-existence of a growth which is capable during life of elbowing itself into clinical prominence by crowding aside important adjoining structures, it is doubtful, as already suggested, whether either the syndrome of Marie or that of Fröhlich would ever have been suspected of its long-secret alliance with an hypophyseal lesion.

It is important to recall that Marie and Marienesco interpreted their postmortem findings of an hypophyseal tumor in a case of acromegaly as an evidence of glandular insufficiency, so that for years experimentalists attempted, without success, to reproduce acromegaly by glandular extirpation. It has always surprised us that no one hit upon the illuminating fact, until the studies of 1908–1909 with Crowe and Homans [4] that animals who survived for long periods after partial extirpations exhibited an unmistakable symptom complex of lessened glandular activity—a picture the reverse of acromegaly.

It will be recalled that cases of acromegaly have been recorded in which not only was there no tumor present, but in which, as was claimed, the gland showed no recognizable histological change. Such cases as these were boldly advanced as a total refutation of Marie's view that the pituitary lesion was the cause of acromegaly, for, inasmuch as the disease could exist without demonstrable (by the existing methods) alterations of the gland, the presence of an hypophyseal growth in some cases must necessarily have been a mere coincidence.

To still further complicate the matter, case reports began to appear describing individuals with actual tumors of the hypophysis who showed, nevertheless, no signs of acromegaly whatsoever, the direction of the recorder's thoughts being clearly indicated by the usual title of "A Case of Pituitary Tumor without Acromegaly"—an indirect argument, as it were, against Marie's doctrine. Thus it was pointed out by Fröhlich that a pre-adolescent tumor of this sort was often accompanied by a definite symptom complex of another order characterized

[4] Experimental hypophyseotomy. Bulletin of the Johns Hopkins Hospital, 1910, xxi, p. 127.

by adiposity and imperfect acquirement of the secondary sexual characteristics.

Fortunately, as has been made clear, experimental investigations have served to reproduce this condition in so far as to make it apparent that this so-called state of dystrophia adiposogenitalis (Bartel) is due in all probability to a condition of functional hypopituitarism. The experiments, furthermore, disclosed the fact that glandular deficiency produced symptoms in adult as well as in pre-adolescent animals, for the latter remained undersized as well as fat and sexually infantile, whereas the adults became fat and showed a tendency for the sexual organs to revert to the pre-adolescent state. We shall see that there are parallel conditions in man, namely, an adult as well as in infantile type of the syndrome described by Fröhlich, just as there are adult and infantile types of myxœdema.

It becomes at once apparent that a gross change in the configuration of the hypophysis itself is no more essential to states of dyspituitarism than it is essential to Basedow's syndrome or to myxœdema in the case of the thyroid. Nevertheless we are unquestionably under deep obligation to these not infrequent glandular enlargements for their aid in having called attention to this stowaway gland, which has been dragged to the light and by "third degree" methods is being questioned as to its motives. And furthermore, in the larger number of cases heretofore recognized, the tumor has actually been such a conspicuous element that for the time being it deserves recognition in the grouping of these conditions.

Clinical states of *increased* glandular activity, though not capable of conclusive experimental reproduction by known methods, are in all likelihood associated either with tumors which actually represent primary hyperplasias or adenomatous processes, or with others which have in some way secondarily aroused functional hypersecretion. On the other hand, clinical states of *diminished* glandular activity which are associated with tumor may be due either to an actual loss of glandular tissue from partial destruction by an infectious or malignant

growth, by vascular disease, hemorrhage, cyst formation, or what not, or, on the other hand, and what is perhaps more common, to the mere "blocking" of its secretory activities from a superimposed interpeduncular growth.

It must be borne in mind, furthermore, that an hypophyseal struma, which at one time has represented a condition of functional hyperplasia, may in the end actually block or destroy the secretory possibilities of the gland. As a matter of fact it may be expected, (1) that in all cases of original hyperpituitarism associated with tumor, the functional end result will be hypopituitarism, and (2) that in many of the cases in which existing hypopituitarism is the striking feature traces at least of an early tendency to hyperpituitarism can be detected. These mixtures of the two syndromes of glandular over- and underactivity are most conveniently designated as dyspituitarism.

THE THERAPEUTICS OF DIGITALIS AND ITS ALLIES[1]

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IN 1775, William Withering, a Birmingham physician, had his attention drawn to a family receipt for the cure of dropsy long kept secret by an old woman in Shropshire; he found it composed of some twenty herbs, of which he concluded that foxglove was the essential one. After trying it in his practice for some months he "ventured to assert" that this herb "merited more attention than modern practice bestowed on it." These were the days of deliberate observation in medicine, however, and it was not until after ten years' careful use of it in his practice that he gave his results to the world,[2] although he had induced many of his friends to use it in the meantime. In his "account of the foxglove," he recognizes its action on the heart,[3] but is chiefly interested in its diuretic effects, and does not associate the latter with the cardiac changes. Withering's work directed immediate attention to the new remedy; I find it given an assured place by Barton, of Philadelphia, in 1798. But its true sphere of action was

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[1] Delivered January 14, 1911.

[2] An account of the foxglove, and some of its medical uses with practical remarks on dropsy and other diseases. By William Withering, M.D., Birmingham, 1785. This work bears the appropriate motto *monumque prematur in annum,* and ought to receive attention as an example of the careful observation of the time; it may be added that it may help to dispel some of the apprehensions of the "cumulative" and other dangerous effects of digitalis which are too prevalent.

[3] "It has a power over the motion of the heart to a degree yet unobserved in any other medicine and this power may be converted to salutary ends" (p. 192).

obscured by its being recommended as a cure for phthisis, although its discoverer denied it any usefulness in this disease from the beginning. Later it slowly resumed its position as the cardiac remedy par excellence, but curiously enough not as a cardiac tonic, but as a depressant. Sixty years after its introduction into regular medicine Pereira, the great authority of his day, recommended it chiefly "to reduce the force and velocity of the circulation," as in aneurism and hemorrhage of internal organs. In his time the new method of experimental investigation of the effects of drugs on animals was in its infancy, but it was soon afterward applied to digitalis by Traube, Schmiedeberg, and others, and the action of digitalis and its allies on the organism was ascertained with certainty in many respects, although it still requires further elucidation. The knowledge of its therapeutic use has also advanced since Pereira's time, and few would venture to recommend digitalis in hemorrhage, and still fewer in aneurism, at the present time. But this advance in the therapeutic applications has scarcely kept pace with that of the experimental investigation. Too often the diagnosis of cardiac abnormality, the discovery of a murmur, is regarded as an immediate indication for digitalis, so that in the narrower sphere of the circulatory diseases, digitalis suffers to-day from the same over-appreciation as was its fate a hundred years ago, when to some minds it appeared to be a panacea for all internal disorders. The advance in the diagnosis of heart disease in recent years, however, gives hope that the sphere of action of digitalis may be more circumscribed, and that we may soon reach a point where instead of "trying digitalis" in every case of heart disease we may be able to define the symptom complex which indicates its exhibition as accurately as that for quinine.

It has long been my feeling that the study of experimental pharmacology should be merely the preliminary to investigation in therapeutics, and I very gladly accepted the invitation of my friend Dr. James Mackenzie to associate myself with him in his clinical researches on the effects of drugs on the circulation. Our final object is to differentiate the types of heart

disease in which digitalis is useful, and I may say at once that some progress has been made in this direction. Incidentally we have carefully noted the effect of digitalis and its allies on the human heart and circulation, with the view that one or the other might be preferable in certain conditions. We are still far from achieving our goal, but a certain amount of information has been acquired, and when your president did me the honor of asking me to address you, I thought that nothing I could offer would be more appropriate to a society that honors the great name of Harvey than a contribution to the therapeutics of the great remedy in diseases of the circulation.

In the experimental laboratory it is found that digitalis affects the heart in several different directions. In the first place, it was recognized early by Traube that the heart is slowed by stimulation of the inhibitory centre in the medulla. Since his time inhibition of the heart has been shown to depress not only the rate, but also the strength of contraction, the conductivity and the tone of the muscle, so that these must be taken into consideration in any account of the changes induced by the drug. On the other hand, digitalis acting directly on the heart muscle increases the strength of contraction, the tone and the excitability. Its direct effects on conductivity have not been accurately determined. The effects of digitalis on the mammalian heart are, therefore, compounded of two influences which in most instances oppose each other. The rhythm is slowed by the inhibition and accelerated by the muscular action, but in moderate quantities in animals the former prevails and the rhythm is slower. The strength of contraction and the tone are reduced by the inhibitory stimulation and increased by the direct action on the heart, and the changes observed are the resultant of these two; in the ventricle the muscular effect prevails and the chamber beats more strongly, and at any rate, in the dilated heart, relaxes less than before the administration. In the auricle, the inhibitory prevails over the muscular factor, and the result is a decrease in the strength of contraction very often, or, it may be, there is little

change to be noted in this chamber from digitalis. In animals the blood pressure rises from contraction of the arterioles which are acted on directly by the glucosides.

In these experiments in animals, the drugs are injected intravenously in order to elicit the effects quickly enough to permit of their being observed within the limits of an experiment of at most three to four hours' duration. In the therapeutic use of digitalis, however, one seldom sees much change from the drug under thirty-six to forty-eight hours. In addition, the dose given experimentally is often very much larger in proportion than that in therapeutic use, and this, together with the rapidity with which it is thrown into the circulation, must cause a much greater concentration in the blood than is induced in therapeutics. Great caution must, therefore, be used in applying the results obtained experimentally in therapeutics, and perhaps this has not been sufficiently appreciated; I cannot cast a stone at any one. But these experimental investigations, at any rate, show that inhibition and muscular action may be elicited in animals by this series, and encourage us to look for these effects in man.

In Dr. Mackenzie's clinic the patients are, of course, examined by his well-known methods, and the course of the disease and the effects of treatment are constantly controlled by graphic tracings in addition to the ordinary clinical notes. Conclusions may, therefore, be drawn with some confidence from a small number of cases. The disorders of the heart were all of such seriousness as to necessitate admission to hospital and detention there, often for months at a time. The treatment with digitalis was almost always preceded by rest in bed for a week or more in order to eliminate as far as possible the error of attributing to the drug changes really due to the general conditions. The disorders of the heart on which we have made our observations may be conveniently thrown into two great groups: (1) Those in which the dominant rhythm of the heart continued to originate in the normal point (Keith-Flack node), and (2) those in which the rhythmogenic function was usurped by some other part of the heart, in our cases by

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the auricle. The effects of digitalis are so different in these two groups that I shall describe them separately.

In the first group digitalis often caused considerable improvement in the general condition, manifested in relief of the dyspnœa or dropsy, or lessened cyanosis, though the benefit was much less obvious than in the second group of cases. This improvement in the general condition was sometimes accompanied by moderate slowing of the pulse, which fell from 90–110, to 70–80 per minute. But this is by no means elicited in every case even when the other symptoms are relieved. Thus, of 18 patients of the first group, only 6 showed any slowing under digitalis, and in one of these it may be questioned whether this was due to the drug. We can safely say that the pulse was slowed by digitalis in only about 30 per cent. of the cases in which the dominant rhythm was of normal origin. In 2 of our 6 cases the pulse was previously very rapid, and it is possible that excessive activity of the rhythmogenic node may favor this effect of digitalis; on the other hand, if this excessive activity arises from fever digitalis often fails to induce slowing. In the other cases in which slowing followed the use of digitalis no feature was observable which distinguished them from those in which no such effect occurred. During the slowing the heart beat continued to bear its ordinary character; in other words, the slowing is due to fewer impulses being emitted by the rhythmogenic area.

The slowing of the heart under digitalis is universally attributed to stimulation of the inhibitory centre in the medulla oblongata, and in fact it bears every resemblance to the effects of such stimulation. Thus the slowing is not uniform, the rhythm varying with the phases of respiration, as it does in normal individuals with marked inhibition. And this respiratory variation may be developed under digitalis in persons in whom it has been previously absent or hardly perceptible. Owing to the development of this feature, the intervals between successive pulse beats may show larger variations after digitalis than before, as Wenckebach has recently pointed out, although perhaps his description tends to exaggerate the significance of this feature.

Further evidence that the slowing is inhibitory in origin is offered by the observation that it disappears under doses of atropine which have no further effect than to prevent the inhibitory impulses reaching the heart. And finally it has been noted that in complete block, digitalis does not slow the ventricle as might be expected if it retarded the heart by direct action on the ventricular muscle, while the auricle continues to be slowed as in the normal heart.

The question arises why digitalis fails in so many instances to slow the heart. We may exclude at once inactivity of the drug because our preparations were assayed pharmacologically and on treating two patients with the same tincture in the same doses, the one may respond by marked slowing, while the other shows no such effect even when the drug is pushed until nausea and vomiting are produced. Either the centre in the medulla is incapable of stimulation in these recalcitrant cases, or it is incapable of holding the heart in check. It is known that the control ordinarily exercised by the inhibitory centre over the heart varies in different individuals and at different ages in the same individual, and evidence is not wanting that the resistance of the heart to the maximal activity of the centre also varies in the same way. In many instances this resistance is great enough to render nugatory the increased activity of the inhibition induced by digitalis; this resistance to inhibition is especially marked in cases of fever, more particularly when the infection is located in the heart muscle itself, as in active myocarditis, in which the irritability of the heart must be considered abnormally augmented.

When digitalis is pushed further in susceptible cases, the inhibition becomes more marked, and obvious irregularities may appear. One of these, like the general slowing, arises from inhibition of the rhythmogenic area—the sinus irregularity, as it has been called. It often occurs without drugs, and consists in alternate phases of slowing and quickening of the heart corresponding to the inspiration and expiration, and is generally attributed to the inhibitory cardiac centre being involved in the stimulation of the respiratory centre; the stimulus

is said to spread to the inhibitory centre. The sinus irregularity of digitalis is sometimes of the same kind, the slowing and quickening corresponding to the movements of respiration fairly closely (Fig. 1). In most cases, however, this does not hold, the phase of slowing recurring at intervals which are much longer than those of respiration. Here one may suppose that the excited inhibitory centre is aroused by stimuli radiating from some other centre than the respiratory, perhaps from the vasomotor area. In any case, in this sinus slowing, as in the regular slowing, the contractions of the heart are normal in character, the only departure being a slower generation of impulses by the rhythmogenic centre. I have already mentioned that careful measurements show that in the ordinary

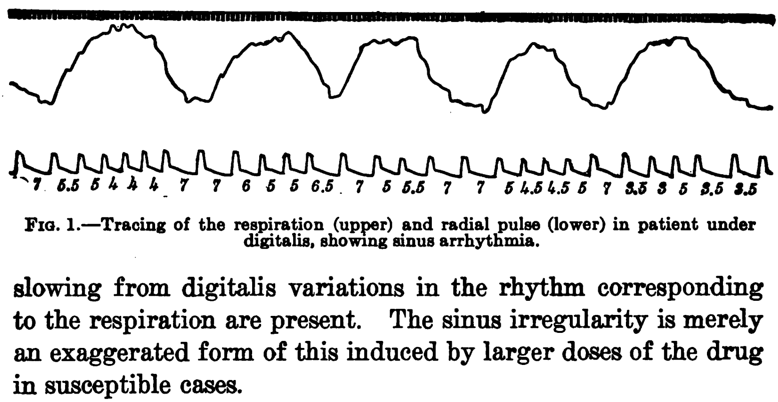

FIG. 1.—Tracing of the respiration (upper) and radial pulse (lower) in patient under digitalis, showing sinus arrhythmia.

slowing from digitalis variations in the rhythm corresponding to the respiration are present. The sinus irregularity is merely an exaggerated form of this induced by larger doses of the drug in susceptible cases.

In other instances in which digitalis is pushed, an irregularity of another kind is developed; the impulses are generated regularly, but fail to reach the ventricle owing to their not being transmitted along the bundle of His. There is, in fact, a partial heart block. The features of this condition have been so fully discussed of late years that it is unnecessary to describe them here. You know how the auricle continues to beat regularly, but every now and then an impulse fails to reach the ventricle and a pulse beat is dropped. This has been noted by a number of observers in the use of digitalis, and has generally been ascribed to the conduction of the bundle having been

reduced by the inhibitory stimulation of the drug. It appears to occur especially in cases in which the transmission of impulses is already impaired by disease, as is indicated by the interval between the auricular and ventricular contraction being much prolonged, but it may be induced by digitalis when no impairment of conductivity was detected before; while on the other hand, Mackenzie gives a case in which the conduction was very slow before digitalis, and did not seem to be further depressed by it. This failure of conduction from the auricle to the ventricle under digitalis is universally ascribed to the inhibitory action of the drug, and in fact, has been seen to disappear under atropine, which removes the inhibitory action of the cardiac vagus. This may seem proof positive and I am

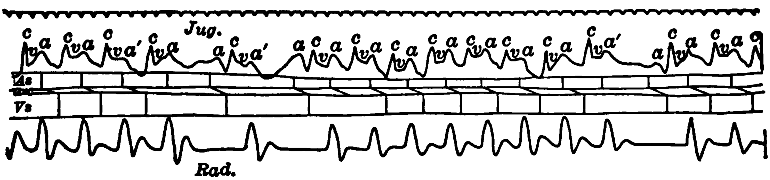

FIG. 2.—Tracing of jugular (upper) and radial pulse (lower) in patient under digitalis, showing occasional failure of the ventricle to contract; a diagram is interposed showing this more clearly. (Mackenzie.)

not prepared to deny that in these cases the inhibition is the potent factor in the irregularity. At the same time, I am not satisfied that it is the only one; for in animal experiments I have seen repeatedly, in fact it is the general rule at a certain phase of the action, that complete block is elicited, the auricles and ventricles beating independently, and yet this block is independent of the inhibitory action for it occurs after large doses of atropine. I should not be surprised to find that in man also the conductivity of the bundle is impaired by direct action on it and the further lowering of its activity by the inhibitory factor induces the block with which we are all familiar. This irregularity is often accentuated at certain phases of the respiration at which the constant inhibitory influence of the drug is reinforced by the stimulus radiating from the respiratory centre.

These two irregularities from excessive inhibition may occur spontaneously in treatment with digitalis. When they are not present, they may sometimes be elicited by further stimulation of the inhibitory mechanism, direct or reflex. Thus slight pressure on the vagus nerve in the neck is often sufficient to demonstrate this action under digitalis. Latent irregularity may also be discovered in some patients by giving them water to sip. Each time water is swallowed the pulse is remarkably slowed and either sinus arrhythmia or partial block occurs. Here, I presume, the excitation of the centres governing swallowing radiates to the centre for cardiac inhibition and arouses it to excessive action. As a measure of the recovery of our patients, we often send them up a flight of stairs and then

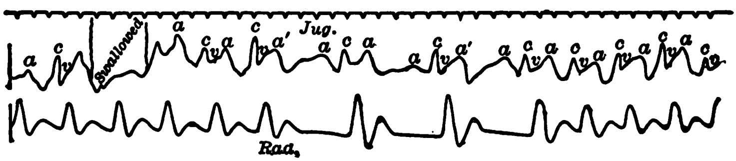

FIG. 3.—Tracing of jugular (upper) and radial pulse (lower) under digitalis in which the rhythm is ordinarily regular, but in which partial block is induced by swallowing. (Mackenzie.)

record the pulse rate immediately afterwards. In a number of cases we have noted that the slowing which follows the initial acceleration of the pulse, went on to irregularity, either sinus or block according to the case; in these there was no irregularity except after this slight exertion.

There can, therefore, be little question that the action of digitalis on the inhibitory mechanism which has been investigated in animals, is exerted in a very similar way in a certain proportion of human patients treated with this drug. In moderate degree the inhibition effect slows the pulse, in larger development it may lead to irregularities or, at any rate, favor their development. The sinus irregularity and the partial block are always preceded and accompanied by slowing. All three features may be developed in the same patient, the sinus

irregularity occurring at one time and block at another.[4] I may add that I elicited and described the phenomena now known as sinus arrhythmia and block by digitalis in the dog many years ago and demonstrated that they were inhibitory in nature. That they occur on stimulation of the vagus in animals is common knowledge.

When we come to look for evidence of the direct action of digitalis on the heart muscle in these cases the position is a much more difficult one, for our present methods of heart examination give very inadequate information of changes in the contractility and tone. I may remark in passing, that here history is repeating itself, for direct proof of the inhibitory action of digitalis was available in experimental investigations on mammals many years before it could be shown to act on the muscle directly. The essential feature of digitalis action in animals in small doses is the stronger beat of the ventricle. In patients, this is difficult to demonstrate. It is true that the apex beat may be considered stronger and the pulse beat larger, but no method of measuring these satisfactorily has yet been devised. And even if this were possible, another difficulty has to be faced; for the heart is often slower, and this in itself would tend to strengthen the contraction of the heart and would increase the stroke of the pulse. So that it is impossible at present to demonstrate directly that the contractility of the heart muscle is increased. Another feature observed in animals experimentally is the lessened relaxation in dilated hearts, and there seemed some hope that this might be demonstrable clinically. But in a number of cases in which we attempted to satisfy ourselves on this point, we could make out no change

[4] The fact that the slowing, the sinus arrhythmia and block are frequently seen together while the last two arise from different parts of the heart, suggests that in these patients the abnormality consists in unusual sensitiveness of the inhibitory centre rather than in a low degree of resistance of the heart to inhibition. For there is no reason why both the rhythmogenic centre and the bundle of His should so often show the same variations. And this would imply that the absence of slowing in most cases is due to failure of the inhibitory centre to react to the drug.

in the area of cardiac dulness, even when digitalis improved the general condition quite satisfactorily. And the apex beat did not move inward. I do not deny that in some cases these alterations in the physical signs are observable, but in our experience such a change is not by any means always apparent, even when digitalis is exercising its full beneficial effects. In a certain number of cases, however, indirect evidence of the action on the heart muscle may be offered. Thus in a number of patients digitalis failed to reduce the pulse rate or to induce any symptom which could be ascribed to the inhibition; yet in these cases, the embarrassment of the breathing became less, anasarca disappeared and the general condition seemed im-

FIG. 4.—Tracing of radial pulse under digitalis, showing extrasystole after each normal pulse movement (continuous bigeminus).

proved, and this relief must, therefore, be attributed to the only other factor which we know—the muscular action. Similarly, in some cases in which helleborein was given (in ½-grain doses t. d. s.) the pulse was not slowed, and no other symptom of inhibition was elicited, but throbbing of the heart was complained of by some patients, and this also seems to indicate change in the muscular contraction independent of the inhibition. I allow that this evidence is very unsatisfactory compared with that given for the inhibitory action, but more adequate proofs can only be expected when more satisfactory methods of measuring the strength of the cardiac contraction are available. At present, we can measure only the rhythm.

Another feature which occurs in animal experiments is the increased excitability of the heart muscle, which makes itself apparent by a tendency to premature contractions or extrasystoles in the ventricle, or auricle and ventricle. These we have

very often elicited in patients under full doses of digitalis in the form of more or less frequent extrasystoles (Fig. 4). This augmented irritability is unquestionably the result of the direct muscular action of the drug, for, though its occurrence may be favored by slowing of the heart, it often arises in patients who do not present this feature in marked degree. Wenckebach states that extrasystoles may disappear under digitalis medication, but this form of irregularity is so capricious in its appearance that I am not disposed to regard digitalis itself as curative. Another feature which has repeatedly occurred in our cases, although more rarely, is pulsus alternans, in which the pulse beats and also the apex beats are alternately large and small without any difference in the interval between them. This form occurs especially after an extrasystole or after a pause of some duration (sinus slowing) and then gradually becomes less marked and disappears. It is met with in untreated cases of heart disease and is generally regarded as evidence of a serious reduction in the contractility. The most satisfactory explanation is that of Wenckebach, who regards the weak beat as the result of the prolonged activity of the preceding strong contraction. It may, in fact, be regarded as a condition in which the recovery of energy by the heart is retarded, while the dissipation of energy during the contraction is not changed to the same extent or perhaps is unaltered. It may be suggested that digitalis, by increasing the contraction power of the heart without a corresponding effect on the restorative function, may occasionally tend to favor the development of this abnormality in hearts which are predisposed to it.

Turning to the second group of cases, those in which the node of Keith and Flack has lost its leadership, and the rhythm arises from some other part of the auricle, we find that digitalis has a much more striking effect. In fact it may be questioned whether it would have attained its position as *the* cardiac remedy had it not been for its efficacy in this condition, in which its exhibition is often followed by a truly wonderful improvement. The commonest form of this abnormal rhythm is that known as auricular fibrillation, and there is every reason

to infer that a number of Withering's cases were of this description.

In this condition, which has long been known in animals, the auricle is distended and a continual tremulous movement occurs in its fibres, but there is no coördinated contraction, the fibres moving quite independently of each other. Irregular impulses are constantly discharged to the ventricle, which responds with a very rapid and quite irregular rhythm. In man, the condition was first defined by Mackenzie's observations on it, and is characterized by extreme irregularity of the pulse and by the absence of all signs of auricular activity. Its true pathology has only been determined recently. In our cases of this

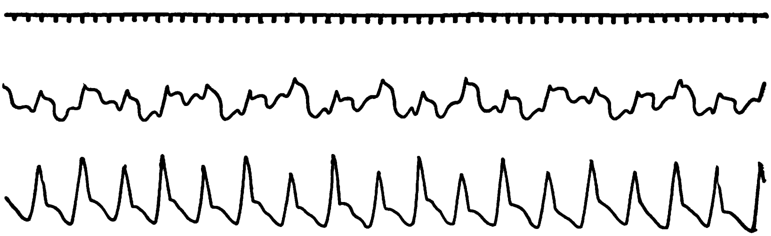

Fig. 5.—Tracing of radial pulse and apex (upper) under digitalis, showing alterations in artery and apex.

condition, the exhibition of digitalis was generally followed by rapid improvement. It is true that it did not restore the normal rhythm, but the symptoms of heart failure disappeared much more rapidly than in cases of the first group in which the normal rhythm was preserved. And coincidently with this improvement in the general symptoms the rate of pulse fell from 120–150, to 60–70 or less.[5] The pulse remained irregular, but the iregularity was less marked (Fig. 6), and the beats were much stronger; the dyspnœa was relieved and the other symptoms of distress underwent marked improvement. The patient was often able to return to his work if it was not of an exhausting nature.

[5] To ascertain the heart rate with even approximate accuracy in these cases, a record of the pulse must be taken or the heart sounds counted.

The most striking objective symptom was the fall in the pulse rate, which offered a marked contrast to that observed in the first group of cases. In these, in which the rhythm arose from the normal position, only about 30 per cent. of the cases reacted to digitalis with slowing, while 11 out of 12 of our cases of fibrillation, or 90 per cent., showed unmistakable slowing. In the twelfth there was fever present and no slowing occurred. The pathology of the condition suggests that this slowing is due to fewer impulses reaching the ventricle

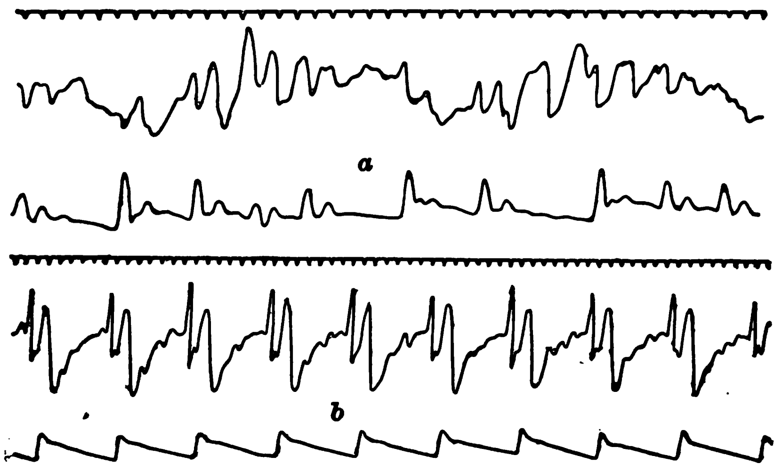

FIG. 6.—Tracing of the jugular (upper) and radial pulse (lower) in a case of fibrillation of the auricle (*a*) before treatment, (*b*) after treatment for some weeks with digitalis.

from the auricle. The condition of the last chamber is unchanged as far as our means of observation teach us, and there seems no other plausible explanation of the fall in the pulse rate than a diminution in the impulses passing the bundle of His. Moreover, in some rare forms of auricular delirium the pulse though completely irregular is not accelerated, and here there is reason to believe that the impulses from the auricle are partially blocked in their passage to the ventricle; in these cases digitalis does not slow the heart nor cause any marked improvement. Wenckebach has shown that stimulation of the inhibition by pressure on the vagus in the neck in auricu-

lar fibrillation induces the same slow pulse as occurs under digitalis, and in a case in which this procedure was ineffective, digitalis also failed to improve the rhythm. One is, therefore, tempted to attribute the remarkable effects of digitalis in the ordinary forms of auricular fibrillation to its inducing a partial block of His's bundle[6] and thus relieving the ventricle from the continual bombardment with impulses from the auricle; that is, the digitalis action here would appear to be pure inhibition. And this explanation is supported by our experience in a number of cases in which the distress disappeared as the pulse fell, and when the treatment was given up for a few days the pulse accelerated, and almost step by step with the acceleration of the pulse, the dyspnœa returned.

In several cases the question has been further tested by the injection of atropine in sufficient quantities to put the inhibition out of action. For example, in a case of fibrillation in which the pulse was originally 150–170, but in which it had been reduced by digitalis to 60–70 per minute, $\frac{1}{50}$ grain atropine sulphate hypodermically accelerated the pulse to about 120. This seems to suggest that the inhibition was a large factor in the effect, though not the only one, for though the pulse was much accelerated by atropine it did not regain its original rate. In another case in which the original rhythm was about 150 per minute, it was reduced to about 50–60 by large doses of digitalis (15 minims four times a day). The patient left the hospital and continued to take digitalis as he felt the need of it, gradually reducing it until after some months he found that his pulse remained slow without the drug, and he only took about a drachm when it began to accelerate, about once a week. Yet on giving atropine at this time the pulse at once rose to 150 per minute, that is, to its original rate. In this case there seems no question that the conduction was re-

[6] Another possible interpretation may be suggested: In auricular fibrillation inhibition makes the tremor of the auricle finer in character, and perhaps the impulses transmitted to the bundle of His are weaker and thus fewer of them reach the ventricle. Digitalis may, therefore, slow the ventricle by weakening the impulses from the auricle rather than by increasing the difficulties in their passage.

duced permanently by inhibition or, rather, that the inhibition had gained a permanent control over the heart, which was absent before the digitalis was given. Whether this is due to the prolonged inhibition to which the heart had been subjected by digitalis in the hospital, or whether the nutrition of the organ had been altered by some other effect of digitalis, *e.g.*, on the heart muscle, I am unable to say. And the fact that our methods give no sufficient indication of the changes in contraction power must render us careful in the interpretation of the symptoms. But I think I am safe in stating that we have no definite evidence that digitalis exerts any action apart from the inhibition in these cases of auricular fibrillation and that the proved fact of inhibition explains all the features observed.

When digitalis is pushed in auricular fibrillation it often reduces the rate still further to 40–50 per minute and induces "coupled beats;" that is, each beat of the ventricle is followed at a very short interval by a second weak contraction and then by a long pause (Fig. 7). The electrocardiograph indicates that the first strong contraction originates in the normal way by an impulse reaching the ventricle from the bundle of His, while the second weak one is a true ventricular extrasystole. In one of our cases in which "coupled beats" were present, atropine accelerated the rhythm of the ventricle by improving the conduction in the bundle and as the rate increased the coupled beats became fewer and finally disappeared. This suggests that the irregularity was at any rate, in part the indirect result of the slow rhythm which allowed the irritability of the ventricle time to develop, and that here the extrasystole resulted indirectly from the inhibition.

In animals I have often observed auricular fibrillation occur from very large quantities of digitalis from excessive stimulation of the auricle muscle. It is of interest to note that in two cases we have met with the same phenomenon in patients. In one of these digitalis had been given for some time without slowing the pulse which maintained a rapid regular rhythm. Suddenly the rate fell to about 70 and the heart became quite irregular. All evidence of auricular activity disappeared—

no *a* wave in the jugular pulse, disappearance of a presystolic murmur which was formerly present—in fact a typical picture of auricular fibrillation under digitalis was presented. With equal suddenness the fibrillation disappeared and the former rapid normal rhythm was restored when the drug was abandoned.

Here, it might be supposed, is certainly an instance where the muscular action was the cause of the irregularity, for the irritability of the heart muscle is augmented by the direct action and culminates in fibrillation. I would not deny the explanation, but I may remind you that fibrillation may occur from inhibition, so that it is possible that here also the action is central.

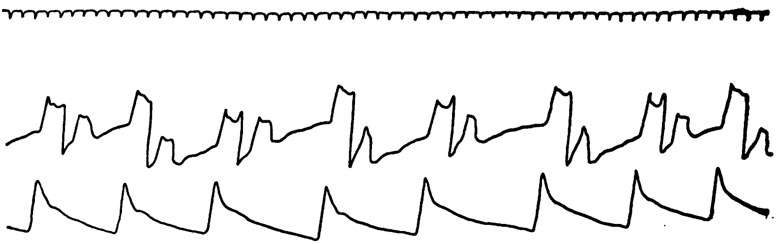

FIG. 7.—Tracing of apex beat (upper) and radial pulse (lower) in fibrillation treated with large dose of digitalis. In the apex tracing an extrasystole is seen to follow each normal beat (coupled beats or continuous bigeminus). These are too weak to cause a pulse in the radial.

Apart from auricular fibrillation we have met two cases in which the rhythm was given by the auricle, but in which it was quite regular though extremely rapid. In these digitalis reduced the rhythm quickly and brought relief from the dyspnœa and other symptoms. In one of them the slowing was interrupted by short phases of what seemed auricular fibrillation, but these became less frequent as the heart rate fell to the normal. The slowing of the pulse in these cases was comparable to that observed in cases of auricular fibrillation, and the question arises whether it was brought about in the same way by a diminished conduction of impulses from the auricle to the ventricle, or whether the actual discharge from the abnormal point in the auricle disappeared under the inhibitory action of the drug.

In any case the efficacy of digitalis and its allies in disorders of the heart arising from auricular hyperactivity is very striking, and raises the question whether the chief usefulness of the drug does not consist in its protection of the ventricle from the too exigent demands made on it by the auricle.

I have given you some instances in which the inhibitory factor seems to play an important, perhaps a dominant part, in the effects of digitalis in therapeutics. Let us remind you of one in which the muscular action alone is desirable. In partial or complete block the indications are to accelerate the ventricle and relieve the symptoms of arrest of the circulation which culminate in the Stokes-Adams syndrome. Bachmann, I think, was the first to utilize the muscular action of digitalis for this purpose, but it might be preferable to employ a member of the series without its inhibitory action, and in future cases I would suggest the use of helleborein or of a mixture of helleborein and digitalis.

The question as to the respective rôles played by the inhibitory and the muscular factor in digitalis is not of theoretical interest only. For if the therapeutic effects are due to inhibition only, they might be elicited by other drugs which do not possess the unpleasant effects on the alimentary tract which present themselves under digitalis. On the other hand, if the muscular action is the essential factor, it might be possible to discover a drug possessing this without the inhibitory effects. In any case it seems desirable that we should be able to vary the proportions of these two factors, for there is no reason to suppose that digitalis presents the exact combination which is suitable in all cases. We, therefore, have treated a number of cases of heart disease with aconite and aconitine, which is reputed to slow the human heart by stimulation of the vagus centre, but has not the digitalis action on the cardiac muscle. But Dr. Price, who has carried out this investigation, finds that aconite does not deserve its reputation in this respect, for it failed to slow the heart even when pushed until unpleasant symptoms appeared; yet these cases afterward responded to digitalis by slowing. On the other hand, we have tried

helleborein, which possesses the muscular effect of digitalis without its action on the inhibition, but the results were not satisfactory, for although no slowing occurred no definite improvement followed in our cases. This may be partly owing to the fact that helleborein tends to cause diarrhœa when given in adequate doses, and we intend to continue its investigation further.

These experiences have led us to make a systematic comparison of the members of the digitalis series in therapeutics. As yet we have taken up chiefly the three common members, digitalis, strophanthus, and squills, and these we have used in the form of the tinctures, as this seemed to be of more importance than the pure principles, which are comparatively seldom employed. Our method is to give digitalis tincture first and push it until it fails to induce further improvement and unpleasant symptoms arise; then all medication is suspended for ten days or more and the patient very often relapses to his former state. Tincture of strophanthus is then given in the same way, again an interval is allowed and finally squills is prescribed. The order is not always the same of course. We have made some interesting observations, for the details of which I must refer to a paper by Turnbull which will appear soon from Mackenzie's clinic. Among these I may mention that we find the tincture of digitalis (B. P.) to be nearly twice as powerful as that of squills or strophanthus, although they have been used in the same dose for many years.[7] Both the therapeutic effects and the untoward symptoms of squills or strophanthus are only elicited when they are given in nearly twice the dose of digitalis.

In reading Withering's account of his treatment one is

[7] These relations hold only for the tinctures of the B. P. The tincture of strophanthus (U.S.P.) is four times as strong as that of the B.P., and according to our results the efficient dose of this would be about one-half that of the tinctura digitalis (U.S.P.) and this is the proportionate dose actually suggested in the U.S.P. The tincture of squills (U.S.P.) on the other hand, is only one-half the strength of that of the B.P., and our results would suggest it being given in three or four times the dose of tinct. digitalis U.S.P.

struck by the fact that he always induced vomiting as soon as possible and then continued his treatment with smaller doses. We have not gone so far as the pioneer, but we have found again and again that the best results are obtained only by the largest doses which can be given without gastric and intestinal symptoms. The dose which we find advisable is a dram to a dram and one-half per day of digitalis tincture, or a dram and one-half to two drams of tincture of squills or strophanthus (B. P.). This, of course, is twice as much or more than is ordinarily prescribed, and I must confess that not infrequently there arises nausea and occasionally vomiting, or sometimes severe headache from the higher dose. But on the other hand, we have patients taking a dram of digitalis per day for months and sometimes going to work under this régime. There is no question that digitalis and its allies are too much feared by the medical profession. The "cumulative" action has been held over us as a bogey for so many years that many prescriptions contain this valuable drug in harmless and useless doses. *Non nocere* is a good rule, but *prodesse* is a better, and tincture of digitalis in two-minim doses three times a day approaches the line of purely expectant treatment.

As regards their relative usefulness there is little difference between these three drugs. We get the same beneficial results and the same undesirable ones as regards the heart, and a patient who shows symptoms of heart block under digitalis, suffers from the same irregularity under squills or strophanthus, while when extrasystole is induced by one it follows from all. Strophanthus is often said to have less tendency to raise the blood pressure than digitalis, but in our patients neither induces any rise of pressure and in some both cause an equal fall. There is, on the whole, rather less tendency to headaches, nausea, and vomiting under strophanthus and squills than under digitalis, but the difference is not very marked and headache is caused in some cases by all three. Slight diarrhœa has followed the use of squills more than the others, and strophanthus also tends to cause this more than digitalis. This tendency to diarrhœa is still more marked from the use

of helleborein and apocynum which we have begun to work upon.

In animal experiments, one of the characteristic effects of digitalis medication is the rise of blood pressure, which arises in part from the heart action, in part from a constriction of the arterioles. In patients the blood pressure is rarely augmented by digitalis and may in fact fall, as the general improvement sets in. This is, I think, due to the much greater efficiency of the vasomotor mechanism in man, which has been developed to permit of his assuming the erect attitude. As the heart fails in disease, the organ which first suffers is the brain; but anæmia of the brain is promptly remedied by constriction of the abdominal vessels, by which the blood is again directed to the head areas. If, now, digitalis is given and the heart action improves, the first effect would be to increase the blood supply of the brain, and this leads the sentinel in the medulla to relax its vigilance, the mesenteric vessels are allowed to relax and the improvement in the heart may thus be coincident with lowered blood pressure. In our experience there is no significant increase in the urine except when dropsy is present.

In the course of this investigation I have been struck by the small amount of accurate knowledge that we possess as to practical therapeutics. My experience has been almost exclusively in the laboratory and perhaps I have expected too high a standard in the clinic, but in this field of cardiac tonics alone I see an endless vista of questions to be solved in the clinic if only accurate observations are available. There seems to me to be no field in which painstaking work is more required and in which the prospects of success are more promising than in clinical therapeutics. I would commend the cultivation of this study to any one who wishes to add to the general store of medical knowledge and at the same time to devote himself to some line of work which will bear upon his own future work. But we have enough of inaccurate therapeutics already; what is needed is not a statistical compilation, but an accurate study of each individual case and a careful and, if you will, an experimental investigation of each feature presented.

THE CHEMISTRY OF THE PROTEINS*

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THE recent advances made in our knowledge of the chemistry of the proteins have attracted wide attention and excited much interest on account of the important connection which they have with many of the fundamental problems of physiology and biochemistry. These newer discoveries have been the subject of many excellent reviews and are now familiar to most of those who are interested in the various branches of biology. On the other hand, there are aspects of protein chemistry which have received but little notice during recent years, although they have an important bearing on the application of these discoveries to physiological problems. These seem well worth bringing before you this evening.

Attention has of late been largely centred on the salient features of the constitution of the proteins as revealed by the brilliant work of Fischer and of Kossel, and many elaborate attempts have been made to apply the ideas suggested by their discoveries. These have at last given us a conception of the constitution of these substances which, while far from complete, has been of the greatest help in dealing with chemical and physiological problems in which the proteins are involved.

The two most important of these new discoveries in protein chemistry are that the proteins consist essentially of combinations of amino-acids joined with each other by a union between the carboxyl group of one with the amino group of another, and that many of the various forms of protein differ widely in their constitution. That the proteins are composed, at least in large part, of combinations of amino-acids can be accepted as proved by the fact that the only substances which have been isolated from the products of their complete decomposition are amino-acids, and a small proportion of ammonia.

* Delivered February 4, 1911.

That these amino-acids are united in the way just stated is proved by the presence among the products of partial hydrolysis of combinations of amino-acids which are identical with synthetic products of known constitution. Whether those amino-acids which are now obtained from the proteins are their only constituents is still undetermined, for in no case, if we except one or two of the protamines, have the recovered products of hydrolysis been even approximately equal to the amount of protein which yielded them. All who are familiar with the methods employed in making these analyses have regarded the values obtained for many of the amino-acids as minimal, but some of these, as, for instance, those for glutaminic acid and tyrosine, have been considered, notably by Abderhalden,[1] as approximately correct. So long as much of the protein cannot be accounted for in products of definite character, uncertainty will prevail as to whether many of the apparent differences between the individual proteins are in fact real or only due to imperfections in the analytical methods, and the uses to which these analyses can be applied will be very greatly restricted. Thus to give a concrete example: from gliadin, which constitutes a large part of the protein of wheat, upwards of thirty-five per cent. of glutaminic acid can easily be isolated, whereas from milk casein, under similar conditions, only about eleven per cent. can be obtained. Since about one-half of the products of casein are as yet unknown it is quite possible that among these is a quantity of glutaminic acid that, for some undiscovered reason, is held in solution, and this may be quite sufficient to make up for the apparently great difference between gliadin and casein.

We have now come to the point where the chemical individuality of our protein preparations is a matter of much importance for future studies, both chemical and physiological, and it will be well to review briefly the data which can help

[1] Abderhalden and Samuely: Zeitschrift für physiologische Chemie, 1905, xlvi, p. 196.

us to form some conclusions concerning this question. As this is the logical starting point for a discussion of the chemistry of the proteins I will take it up first.

A large number of protein substances are now on record to which special designations have been given, but about many of these we know at present comparatively little. To a few of them much attention has been directed, but unfortunately many of those who have worked with them have had little experience with such substances and their efforts have led to so much confusion that it is almost impossible to form any estimate of the value of the recorded data.

As the animal organism consists for the most part of protein, and as each individual protein occurs associated with many others, it has been especially difficult to isolate products of definite properties from such tissues. The animal proteins are the ones with which the majority of those interested in protein chemistry are familiar, and it is not surprising that the definite character of our so-called individual proteins is regarded with much more than suspicion. Very little really serious work has been done with any protein of animal origin except ovalbumin. This is the only one which has been subjected to careful fractional crystallization, whereby the constant chemical and physical properties of successive fractions have been established. In regard to crystallized serum albumin less is known than of ovalbumin.

In the case of the hæmoglobins the matter stands even worse, for no extensive study of the products of fractional crystallization has yet been made, and some of the recorded data as to ultimate composition are manifestly wrong in consequence of analytical blunders. These, however, are still quoted as evidence of the uncertain composition of this substance, and it seems to be generally believed that it is impossible to make two preparations of these beautifully crystallizing substances which are alike.

The seeds of many plants afford the best material from which to obtain preparations of definite character, for in these we find a relatively large proportion of reserve protein, which

is in a sense the excretory product of the protoplasm of the cells of the ripening seed. This reserve protein is far more stable than that from animal tissues, and usually can be subjected to extensive fractional crystallization, or precipitation, without showing any detectable change in properties. As such protein has the characteristic structure shown by the animal proteins it is the best material now at our disposal for a study of the chemical and physical characteristics of proteins in general, and apparently furnishes the best preparations for experimental studies of nutritional and other physiological problems. The knowledge gained by such study will to a large extent be applicable to proteins of animal origin.

In regard to the chemical individuality of any of the proteins nothing definite can be said, but there are good reasons for believing that many of those from seeds consist of but a single substance, as the methods which have readily shown the presence of two or more proteins, in preparation from some seeds, have not given the slightest evidence of an admixture in those from others.

The crystalline globulin edestin, which is obtained from hemp-seed, has been the subject of a most extensive fractionation under a great variety of conditions, and has yielded fractions which have not shown any differences which exceeded the limits of error of observation in ultimate composition, in the partition of nitrogen, or in specific rotation.[2] These facts are important, for edestin yields on hydrolysis just as complex a mixture of amino-acids as do any of the proteins yet analyzed, and hence affords no basis for the belief that the individual proteins, as we now know them, are mixtures of relatively simple polypeptides, each containing but a few of the amino-acids. It is impossible after my experience to believe, as Fischer suggests, that many of the seed proteins are mixtures of several substances of simpler constitution. If this view were correct, it would seem improbable that a substance which crystallizes readily in definite form would resist all possible

[2] Unpublished results obtained by the author.

efforts to break up the mixture to at least some extent. This question deserves still more critical study, and such work is now in progress in my laboratory.

I have gone into this question of the possibility of obtaining preparations of probable chemical individuality, because there is a wide-spread belief, largely founded on the indefinite character of the preparations of animal origin, that it is impossible to make protein preparations which are worthy of the consideration of the chemist.

To secure a few well characterized proteins is important to the future progress of protein chemistry, for our next task is to subject some of the best defined of these to exhaustive investigation of all their properties. It seems to me that only in this way can we ultimately acquire definite information which can be applied to proteins in general and thus obtain a secure foundation for physiological experiments and conclusions. In the process of differentiating the proteins the efforts of the earlier investigators were directed toward discovering similarities between those of different origin, for it was assumed that there were in nature only a comparatively small number of forms. As the means for differentiation were developed it gradually became apparent that the number of individual proteins was very great. The recently discovered precipitin and anaphylaxis reactions made it probable that an almost infinite number of chemically distinct forms occur in different animal and vegetable tissues, but the evidence was not conclusive that these reactions were actually due to the proteins contained in the different fluids and extracts which had been used in obtaining these reactions until Wells reported his anaphylaxis experiments, made a short time ago with carefully recrystallized ovalbumin. Some experiments with plant proteins, just published by Wells and myself, fully confirm this conclusion, and indicate very strongly that the specificity of the anaphylaxis reaction is intimately connected with the structure of the protein.

One series of our experiments are of direct interest in this connection, as they indicate that this reaction can be used

to determine the relation of one protein to another, when these are so nearly alike that differences between them cannot be recognized by a most careful comparison of their physical and chemical properties. Preparations of globulin from the seeds of hemp, flax, squash, and castor-oil plant are obtained which are so nearly alike in ultimate composition, crystalline form, solubility and physical properties that only the most minute examination has revealed any differences whatever between them. We found that while all these proteins showed a strong anaphylaxis reaction, only those from the flax-seed and castor-bean showed any tendency whatever to react with one another, and between these the reaction was of a doubtful character. On the other hand, gliadin from rye reacted strongly with gliadin from wheat, a result in accord with the fact that by chemical or physical means no differences have been detected which were sufficient to indicate that these gliadins were different substances. Likewise legumin from the pea reacted with the apparently identical legumin from the vetch. No reactions were obtained between proteins of distinctly different structure nor between those from seeds botanically unrelated.

From these facts it is evident that structural differences exist between very similar proteins of different origin and it is interesting to note that chemically identical proteins apparently do not occur in animals or plants of different species, unless these are biologically very closely related. In this respect the proteins are in marked contrast to the other constituents of plants and animals, for not only do identically the same sugars and fats occur in many species of plants and animals, but many of these are common to both forms of life. It thus appears that the chemical constitution of the proteins is closely connected with the biological relations of the forms of life which produce them, and that the morphological differences between species find their counterpart in the protein constituents of their tissues. A similar differentiation has recently been made by Reichert and Brown[8] on the basis of the crystalline form of

[8] Reichert and Brown: Carnegie Institution of Washington, Publication 116, 1909.

the hæmoglobins, since the measured angles of the crystals show close generic relations.

We have thus far merely considered the fact that differences exist between the proteins, but for the present problems of physiology the extent of these differences is of much more importance, for it is only within the last ten years that we have come to realize that the differences in the structure of many of the proteins, especially some of those extensively used for food, are so great as to require a complete change in our views of digestion and assimilation.

The first observation of an important qualitative difference was made long ago on gelatin, which by its failure to give more than a feeble Millon's reaction, was known to contain no tyrosine. This protein was, however, not regarded as a typical one, but was assigned to the group of the albuminoids containing those protein-like substances which compose the greater part of various specialized tissues having little, if any, physiological activity. Since none of these substances were regarded as capable of supplying protein to the tissues when taken as food, no special importance was attached to the fact that gelatin was deficient in one of the common constituents of food proteins, although it was known that it was capable of replacing a part of the protein in a maintenance diet.

Attention was first directed in 1900 to the chemical constitution of food proteins, in their relation to nutrition, by Kossel and Kutscher,[4] who found that the alcohol-soluble proteins, which form a large part of the protein of wheat and maize flour, contained no lysine. These had long been used with success in feeding both men and animals, and the question of their nutritive relations was at once raised. I also found that zein, the alcohol-soluble protein of maize, which Kossel had found to yield no lysine, fails to give the Hopkins-Cole reaction, and therefore contains no tryptophane.[5]

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[4] Kossel and Kutscher: Zeitschrift für physiologische Chemie, 1900, xxxi, p. 165.

[5] Osborne and Harris: Journal of the American Chemical Society, 1903, xxv, p. 853.

In respect to quantitative differences between food proteins a series of determinations of the partition of nitrogen, made by Harris and myself[6] in 1903, showed that such wide differences in their structure existed that if these had equal values in nutrition a very considerable change in constitution must be effected in the process of assimilation which would involve much more elaborate synthesis than, at that time, was supposed to take place in the animal organism. A striking discovery, made in my laboratory, which showed to what extent quantitative differences might occur in proteins of recognized food value, was that gliadin from wheat flour yielded over 35 per cent. of glutaminic acid.[7] This observation was shortly followed by analyses of the products of hydrolysis of a large number of proteins from many sources, which have given us a general picture of the main peculiarities of most of those which are commonly present in our foods. In these analyses important differences in the proportion of each of the amino-acids have been revealed, especially among the reserve proteins of seeds. Thus through the gradually developed recognition of the fact that our food proteins differ widely in their constitution, entirely new aspects of the problems of digestion and assimilation have been raised, which are still the subject of investigation and controversy.

The physiologist may well ask, are these differences between the various food proteins as great as they appear to be? This question is justified, for, as I have already said, in these analyses hardly more than one-half of the total protein has been recovered in the form of definite products. Although there is no doubt that, in many respects, very considerable structural differences actually exist between many of the proteins commonly used for food, the degree of the accuracy of our present analyses should be considered in detail, so that the actual quantitative value of the determination of each of the amino-acids can be definitely ascertained.

As a critical study of these methods has been in progress

[6] Osborne and Harris: *Ibid.,* p. 323.

[7] Osborne and Harris: American Journal of Physiology, 1905, xiii, p. 35.

in my laboratory for some time, it may be a matter of interest to you to know some of the results already obtained. Not only our own work, but that of other laboratories, has shown that the amount of ammonia yielded by hydrolysis is uniform for each protein, and can be determined with accuracy. The proportion of the protein nitrogen obtained as ammonia has thus been found to be four times greater in some proteins than in others. The amount of arginine, histidine, and lysine can be estimated by Kossel's method with a relatively high degree of accuracy and can be controlled by determinations of the quantity of nitrogen precipitated by phosphotungstic acid under definite conditions. The results of the direct estimations of the basic amino-acids have been found to be very nearly equal to the amount of these substances actually present, and to be strictly comparable with one another. The only uncertainty attaching to carefully made determinations is caused by incomplete hydrolysis of the protein; this can be controlled by estimating the proportion of nitrogen precipitable by phosphotungstic acid, after hydrolyzing with hydrochloric acid for different periods of time.

From the results that we have obtained from a number of food proteins it has been found that the proportion of arginine or histidine is about ten times as great in some as in others. The proportion of lysine varied from none to 6.43 per cent. of these proteins while the lysine, obtained from the crude muscle substance of chicken and halibut, reached nearly 7.5 per cent. of the dry material, indicating that the pure proteins of these tissues yield an even greater quantity.[8]

Tyrosine, which has always been isolated by direct crystallization, can probably be estimated with a reasonable approximation to accuracy, although, according to my experience, it can never be so completely separated from the mixture of the amino-acids as Abderhalden assumes, who has stated that it is possible to thus separate it completely and regards its determination as one of the most accurate of those employed in analyzing the products of protein hydrolysis. If only pure

[8] Cf. Osborne, Leavenworth and Brautlecht: American Journal of Physiology, 1908, xxiii, p. 180.

tyrosine is weighed it seems possible to determine its proportion with a close approximation to the truth, but it is not always possible to determine the purity of the products weighed by some of those who have published such estimations. The amount of tyrosine obtained from food proteins falls between 2 and 4.5 per cent., although as much as 10 per cent. has been obtained from some of the albuminoids.

It has been commonly assumed that glutaminic acid in the form of its hydrochloride can be almost completely separated from the mixture of decomposition products by the method of Hlasiwetz and Habermann. Abderhalden has used this determination in order to show the relations of various proteins to one another, and has stated that the results are more nearly quantitative than those obtained for any of the other mono-amino-acids, with the exception of tyrosine. Confidence in the accuracy of these determinations has been largely founded on the close agreement between different determinations. Thus several investigators independently obtained from 10.5 to 11 per cent. of glutaminic acid from casein, and a like agreement was also obtained with several other proteins. In making these determinations the question whether the protein was completely hydrolyzed or not received little attention, for it was formerly assumed when the products of hydrolysis ceased to give the biuret reaction that the union between the amino-acids had been completely broken down.

Some time ago I found that a considerable quantity of an insoluble product was formed by hydrolyzing gliadin for several hours with twenty-five per cent. sulphuric acid, and that this, on subsequently boiling with strong hydrochloric acid, yielded relatively large quantities of glutaminic acid and cystine. The most direct evidence of the existence within the protein of a highly resistant union between two amino-acids was exhibited by the di-peptide of proline and phenylalanine which was obtained by Clapp and myself [9] from gliadin which had been boiled with 25 per cent. sulphuric acid for many hours. This

[9] Osborne and Clapp: American Journal of Physiology, 1907, xviii, p. 123.

di-peptide was completely hydrolyzed only by heating in a closed tube to a relatively high temperature for some time with strong hydrochloric acid. We thus have every reason to expect combinations of amino-acids which will require very energetic treatment with acids before they can be completely decomposed. These observations have led me to determine the output of amino-acids after prolonged boiling with strong hydrochloric acid, and as a result I have obtained from several proteins much more glutaminic acid than had formerly been isolated from them. Thus after doubling the time of hydrolysis of casein I have recently found, in five closely agreeing estimations, 15.5 per cent. glutaminic acid, or 50 per cent. more than was previously isolated. Zein, gliadin, and edestin have also yielded distinctly larger quantities than after the shorter hydrolytic treatment to which they had been previously subjected.

Before the results, thus far reported, in respect not only to glutaminic acid, but also to all of the other amino-acids obtained by Fischer's ester method can be accepted as final, they must be confirmed by new determinations made after boiling the proteins with strong acids until the hydrolysis is complete.

Heretofore we have had no means whereby we could satisfy ourselves that the union between all of the amino-acids had been broken apart and the acids set free. Sörensen's method of determining this by adding methylene to the amino-nitrogen, and estimating the proportion of free carboxyl groups by titration, does not yield satisfactory results in practice, as the endpoint of the titration is too uncertain. The method recently proposed by Van Slyke [10] for determining the proportion of free amino groups yields good results, and by its use the progress of the hydrolysis should be easily and accurately followed.

Although the yield of amino-acids can be increased by making the hydrolysis complete, the errors incident to the processes employed in isolating them involve losses which contribute

[10] Van Slyke: Berichte der deutschen chemischen Gesellschaft, 1910, xliii, p. 3170.

largely to the deficit shown by the total of the products obtained in definite form. As no data were on record from which these losses could be even roughly estimated Jones and I [11] made a mixture of amino-acids in the proportion in which they had been obtained from zein, and analyzed it according to Fischer's ester method. We recovered only a little more than 80 per cent. of the leucine, about 70 per cent. of the proline and phenylalanine, and 40–50 per cent. of the alanine, valine, and aspartic acid, and none of the serine. In making this test analysis, ill-defined products similar to those observed in the course of an ordinary protein analysis were formed, showing that decomposition of the esters took place during the distillation.

By correcting our analysis of zein for corresponding losses 92.7 per cent. was accounted for as consisting of those amino-acids which are now recognized as protein decomposition products. Hence nearly all of the deficit shown by the analysis of zein may fairly be attributed to analytical errors, for the presence of 0.6 per cent. of sulphur shows that a part of the substance, still unaccounted for, belongs to some sulphur-containing complex, and also a part unquestionably to serine which, we are convinced, must have been present in much larger amount than that isolated. We cannot apply corresponding corrections to the analyses of other proteins until these have been subjected to further careful study, for differences in the constitution of the mixture of amino-acids may lead to losses quite different in extent. From the experience gained in this case, however, the way appears open for further investigations which should give us a more definite conception than we now have of the constitution of some of the more important food proteins.

Proteins which, unlike zein, give a strong Molisch reaction and contain carbohydrates, deserve especial consideration, for in the analysis of these the carbohydrate complex may have a pronounced influence on the results. It is generally assumed

[11] Osborne and Jones: American Journal of Physiology, 1910, xxvi, p. 30.

that carbohydrate complexes form an integral constituent of the molecules of many of the proteins. An amino-carbohydrate has been obtained from ovalbumin, ovomucoid and some of the mucins, but attempts to isolate any such substances from many other proteins have failed. The fact that most protein preparations give the Molisch reaction has led to the assumption that these contain some carbohydrate, but my experience has led me to believe that in most cases this reaction is caused by impurities in the preparation and not by a constituent of the protein molecule. This is certainly true for many of the plant proteins, from some of which every trace of carbohydrate can be easily removed, while from others this can be done only with difficulty.

Of the proteins which contain phosphorus we have two types—the nucleoproteins and the phosphoproteins.

Little need be said concerning the constitution of the nucleoproteins, other than that these are natural or artificial combinations of simple proteins with nucleic acid. The constitution of the nucleic acids has been revealed largely by the brilliant work of Levene and Jacobs,[12] but as these form no part of the protein molecule a discussion of their decomposition products lies outside the scope of this lecture.

The phosphoproteins, which like milk casein or ovovitellin, contain phosphorus, but not nucleic acid, present a wholly different problem, for in these the phosphorus appears to bear some relation to the protein molecule. No non-protein, phosphorus-containing group has yet been obtained from these proteins, and it cannot be assumed that these are combinations of phosphorus-free protein with such a complex, although the fact that the total quantity of decomposition products accounted for in analyzing both casein and ovovitellin is small indicates that this may be the case.

This brief review will have little interest for you unless in connection with it I consider some of the problems which

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[12] Levene and Jacobs: Berichte der deutschen chemischen Gesellschaft, 1910, xliii, p. 1.

have been presented to the physiologist by the recent investigations in the field of protein chemistry.

The most important of these problems have been raised by the discovery that many of the proteins which are extensively used for food differ much in constitution, not only from the tissue proteins of the animal, but also from each other. In consequence of this the older views of protein assimilation have been abandoned, and a multitude of new questions now demand an answer. Since we can no longer assume that the food protein is but slightly changed in the process of digestion before conversion into the animal tissues, we must consider in how far it is decomposed by the digestive enzymes and to what extent a re-synthesis is effected in the process of assimilation.

A number of distinct and independent problems are thus raised, each of which must be settled before these questions can be answered.

First: To what extent is the protein decomposed by the normal process of digestion?

Second: To what extent does the animal synthesize the products of protein hydrolysis?

Third: What is the minimal protein requirement of the animal? In other words, how much of the food protein actually replaces protein waste in the tissues, and how much is burned, without ever being converted into tissue substance?

Fourth: To what extent do intestinal bacteria take part in these processes?

All these questions have been the subject of investigation and discussion, but a conclusive answer has not yet been obtained to any of them.

It has long been known that some of the ultimate products of protein hydrolysis occur in the intestine, although the importance of the earlier observations has only recently been recognized. Thus Koellicker and Müller,[13] in 1856, found leucine and tyrosine there, and later Kühne[14] confirmed their

[13] Koellicker und Müller: Verhandlung der physikalisch-medicinischen Gesellschaft in Würzburg, 1856, vi, p. 507.

[14] Kühne: Virchow's Archiv, 1867, xxxix, p. 155.

observation, but considered these to be by-products of the action of trypsin. As you all know, Kühne assumed that under the action of trypsin the proteins can be decomposed into nearly equal parts, which he called respectively hemi- and anti-peptone, and by the continued action of trypsin the former is completely converted into amino-acids, but the latter is resistant to any further action of this enzyme. He, however, did not believe that in the normal digestion the decomposition of the hemipeptone was carried to the amino-acid stage.

In 1901 Kutscher and Seemann [15] found that protein was normally converted into amino-acids in the intestine and supposed that these served as the material from which the new body protein was constructed. At the same time Cohnheim [16] showed that the intestine contained the enzyme, erepsin, which, although without action on native proteins, converts the proteoses and peptones formed by pepsin and trypsin completely into simpler products, among which amino-acids are abundant. Thus almost at the very time when chemical investigations made it necessary to assume that the food protein is decomposed into amino-acids and new protein reconstructed from these products of hydrolysis, physiological investigations showed that the animal organism was equipped with enzymes able to accomplish this result.

However, it still remains undecided whether decomposition of the food protein, under normal conditions, is actually carried to a complete conversion into amino-acids, for Fischer and Abderhalden [17] consider this to be highly improbable, and suggest that it is possible that, after a certain proportion of amino-acids are split off, a nucleus may remain to serve as a basis for the construction of new protein.

The question of in how far the animal is able to synthesize

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[15] Kutscher und Seemann: Zentralblatt für Physiologie, 1901, xv, p. 275.

[16] Cohnheim: Zeitschrift für physiologische Chemie, 1901, xxxiii, p. 451.

[17] Fischer and Abderhalden: Zeitschrift für physiologische Chemie, 1903, xxxix, p. 83.

protein from the products of protein hydrolysis has been put to a direct test. Loewi,[18] Lesser,[19] Abderhalden and Rona,[20] Henderson and Dean [21] and others have found that not only nitrogen equilibrium but even a distinct nitrogen retention could be obtained by feeding animals with the products of tryptic digestion, but not with the products of acid hydrolysis.

It has been thought that the products of tryptic digestion serve to maintain the animal better than do those of acid hydrolysis because they contain undecomposed polypeptide complexes which may serve as a nucleus to which amino-acids of the proper kind and in the right proportion are added. It has also been supposed that the products of acid hydrolysis fail to maintain the animal because of the destruction of some one, or more, essential constituent of the protein through secondary decomposition caused by the acid, for it is known that such occur, notably in the case of cystine and tryptophane.

The question of the dependence of protein synthesis on the structure of the food protein has been investigated by Abderhalden and Samuely,[22] who sought to detect a change in the glutaminic acid content of the serum albumin of a horse after feeding large quantities of wheat gliadin, from which several times as much glutaminic acid can be obtained as from serum albumin. As no change in the composition of the blood albumin could be discovered they concluded that the composition of the food protein had no influence on the composition of the blood proteins.

The same question was also experimentally tested by

[18] Loewi: Zentralblatt für Physiologie, 1902, p. 590; Zeitschrift für Biologie, xlvi, N.F. xxviii, p. 113, 1904; Archiv für experimentelle Pathologie und Pharmakologie, 1902, xlviii, p. 303.

[19] Lesser: Zeitschrift für Biologie, 1904, xlv, N.F. xxvii, p. 497.

[20] Abderhalden und Rona: Zeitschrift für physiologische Chemie, 1907, lii, p. 507.

[21] Henderson and Dean: American Journal of Physiology, 1903, ix, p. 386.

[22] Abderhalden and Samuely: Zeitschrift für physiologische Chemie, 1905, xlvi, p. 193.

Michaud,[23] who founded his experiments on the assumption that the animal requires for the construction of its body proteins a definite proportion of each of the amino-acids which enter into their constitution. He therefore thought that an animal could be maintained in nitrogen equilibrium by a smaller amount of protein consisting of a mixture of the tissues of an animal of the same species than of protein of distinctly different constitution. He accordingly fed dogs on a mixture of minced dog tissue, and compared the nitrogen balances with those obtained with food mixtures which contained the same quantity of nitrogen in the form of gliadin, edestin, nutrose, casein, dog serum proteins or horse flesh. The results which he obtained were very striking. The minced dog tissues were far more effective in preventing loss of nitrogen than either gliadin or edestin, and distinctly more effective than casein. The differences obtained with dog serum proteins, or with horse flesh, were too slight to lead to any definite conclusions.

The question of protein synthesis by the animal has also been studied by aïd of the so-called "incomplete proteins." The numerous older experiments with gelatin, which lacks tyrosine, have led to the belief that this alone cannot support life, but that when added to a food containing other proteins it is capable of replacing a considerable part of the protein required to maintain nitrogen equilibrium.

Wilcock and Hopkins [24] found that zein, which lacks glycocoll, lysine, and tyrosine, failed to keep mice alive for more than a few days, but that if tryptophane was added they lived somewhat longer.

Henriques [25] fed rats with zein and also with gliadin, which lacks glycocoll and lysine, and found that he could not secure nitrogen equilibrium with zein but could do so with gliadin if fed in sufficiently large quantities. He concluded that probably the absence of tryptophane rendered zein incapable of sup-

[23] Michaud: *Ibid.,* 1909, lix, p. 404.

[24] Wilcock and Hopkins: Journal of Physiology, 1906, xxxv, p. 88.

[25] Henriques: Zeitschrift für physiologische Chemie, 1909, lx, p. 105.

porting an animal, but the absence of lysine from gliadin was not of essential importance.

Definite data concerning the minimal protein requirement of the animal are lacking. In complete starvation a considerable quantity of nitrogen is eliminated which is much reduced if the energy requirements of the animal are satisfied by feeding sufficient carbohydrate. Michaud's [26] experiments have shown that this quantity can be still further reduced by interposing a period of feeding with a minimal quantity of protein and then again feeding with nitrogen-free food, but his experiments left him in doubt as to whether or not the output of nitrogen thus found corresponded to the destruction of tissue actually necessary to maintain the physiological functions of the body. This is of importance in connection with the question of the synthesis of body protein from food protein, for we must know how much of the latter is required for this purpose if we are to interpret the results obtained in feeding animals with proteins of different constitution. From Michaud's results it is evident that only a small part of the food protein commonly consumed is necessary to supply the tissue waste incident to the performance of the purely physiological processes required to support life, and that consequently, under normal conditions, a synthesis of new protein probably occurs to only a small extent.

The part which intestinal bacteria may play in the synthesis of body protein from food protein deserves much more attention than it has received. It is well known that bacteria are present in large numbers in the intestine, for from 30 to 40 per cent. of the fæces may consist of the bodies of these organisms.

Before we can accept as conclusive any of the evidence that has been offered that the animal actually synthesizes tissue proteins from the constituents of its food, we must carefully consider the data just set forth, not only in connection with each other, but also in connection with some other facts which have been recently discovered.

[26] Michaud: *l. c.*

PLATE 1.

1

2

3 4 5 6 7 8

1, An albino female guinea-pig. Its ovaries were removed and in their place were introduced ovaries from a young black guinea-pig. Compare 17. 2, An albino male guinea-pig, with which was mated the albino shown in 1. 3–5, Living young, and 6–8, skins of unborn young of the pair of albinos shown in 1 and 2.

PLATE 2.

9

10

11

12

9. An albino guinea-pig, father of black young like those shown in 11. Compare 9 and 11. 11. A black female guinea-pig and her young. 10. Two of the grown-up young of a black and an albino guinea-pig. 12. A group of four young produced by the animals shown in 10.

PLATE 3.

13

14

15

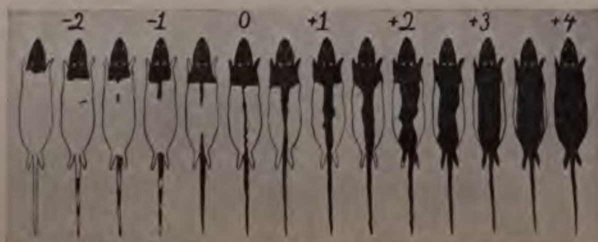
16

13, A dark smooth guinea-pig. 14, A dark rough guinea-pig, the new combination of characters obtained when animals are mated like those shown in 13 and 15 respectively. 15, A white rough guinea-pig. 16, A white smooth guinea-pig, a second new combination of characters, but obtained first among the grandchildren of such animals as are shown in 13 and 15. Other grandchildren are like the respective grandparents (13 and 15) or the parents (14).

PLATE 4.

17

18



19

A

B

C

D

17. A young black guinea-pig about three weeks old. Ovaries taken from an animal like this were transplanted into the albino shown in 1. 18. Diagram showing variation in pattern of hooded rats. 19, *A*, front-feet of normal guinea-pig; *B*, hind-feet of same; *C*, hind-feet of polydactylous guinea-pig; *D*, hind-feet of a cross between *B* and *C*

From Michaud's[27] experiments it is evident that only a small part of the food protein normally consumed is used for the construction of tissue protein, and that consequently when the animal receives an abundant supply of food a deficiency in one or more of the essential constituents of the protein may not become apparent for a long time. Furthermore, such a deficiency may be supplied by the animal's own tissues, for we know that the muscles form a reserve supply which furnishes the necessary protein required during long-continued starvation and also serves for the construction of new tissues, under normal conditions, as shown by the development of the reproductive organs of the salmon.

It is, consequently, probable if the synthesis of the animal proteins is affected by a recombination of amino-acids that the deficiency of any one of these in the food protein will become apparent only when the experiments are carried on for much longer periods than has thus far been done in testing the proteins in respect to their relations to protein synthesis in the animal body.

All the experiments in this direction which have been made with the incomplete proteins are wholly inconclusive because similar experiments made with complete proteins have likewise failed, with the exception of one by Röhmann, in which he used a mixture of several proteins.

The fact that a nitrogen balance is not obtained in such experiments is no evidence that the fault lies in the constitution of the protein, as I shall soon show. Also a retention of nitrogen obtained for a time with a protein or its decomposition products is not evidence that these have been utilized in the construction of new tissue.

That the products of tryptic digestion maintain the animal for a short time while the products of acid hydrolysis fail to do so is not evidence that some essential amino-acid has been destroyed by boiling the protein with strong acid, for the failure of the latter may as well be due to the presence of some

[27] Michaud: *l. c.*

more or less toxic secondary decomposition product, as is indicated by the digestive disturbances noted by all those who have fed such substances.

Michaud's[28] discovery that proteins which, in constitution closely resemble the body proteins, prevent loss of nitrogen from the body better than do proteins which differ widely in their constitution from the body proteins, is the best evidence that we have that amino-acids from the food protein are actually used for the construction of the tissue proteins. This, however, is not wholly convincing, for Michaud's experiments are, in fact, a comparison of the nutritive effect of animal tissue substance with the nutritive effect of a mixture of isolated protein and other substances. It is also to be noted that he makes no mention of the addition of inorganic constituents to these mixtures. It is not surprising, therefore, that he should have obtained the best results with the tissue feeding, for the comparative failure of the isolated proteins may have been due to other causes than differences in their constitution.

In none of the experiments thus far discussed has consideration been given to the possible influence that bacteria may play in the transformations that are required to convert a protein of wholly different constitution from the tissue proteins into the substances which compose the body of the animal. The capacity of these organisms to effect profound chemical changes is quite sufficient to transform a considerable part of the amino-acids which result from digestion of the food protein into forms of totally different constitution. By this means an excess or deficiency of one or another amino-acid may be compensated and the animal supplied with an entirely different combination of amino-acids from that originally fed to it. To what extent such changes occur in the intestine, or to what degree the substance of the bacteria is digested and assimilated, we do not know. The fact that animals in cages on restricted diets are prone to eat their own fæces indicates that they thereby secure some element of food which they crave, and is sug-

[28] Michaud: *l. c.*

gestive that the bacteria may have more influence in feeding experiments than has heretofore been supposed.

After all that has been said and written concerning the synthesis of the body protein by recombination of the amino-acids from the food proteins it must not be forgotten that in the process of digestion the amino-acids themselves may be deaminized and converted into wholly different substances, and that from these new products the amino-acids required by the animal may be later reconstructed.

Many facts have indicated that this may happen, but the most important indication has very recently been given by Embden and Schmitz, who have found by perfusing the excised liver with blood to which pyroracemic acid or lactic acid had been added that alanine is formed. They also obtained tyrosine and phenylalanine after adding oxyphenylpyroracemic acid or phenylpyroracemic acid respectively.

In reviewing the literature of feeding experiments made with a view to determine the possible synthesis of protein by the animal, it is evident that these have been much too brief and have been made without sufficient consideration of many important factors. In most of these the ages of the animals have not been given, in others the isolated proteins have been commercial products of doubtful purity or have been prepared in the laboratory by hasty methods which do not yield products of definite character; no sufficient consideration has been given to the requirements of the animal for inorganic constituents, and none whatever, so far as I can find, to changes caused by intestinal bacteria in the constitution of the nitrogenous elements of the food. Each of these factors may have an important influence and must be the subject of special investigation before final conclusions can be reached. Such investigations must be made along many lines before a foundation can be secured from which conclusions of value can be drawn. These problems are among the most complex that have been presented to the physiologist, and it needs but a little reflection to show that a solution can be reached only by long-continued and patient work.

A beginning in this direction was made about a year and a half ago by Mendel and myself with the hope that in time we may secure some data which may ultimately be of help in solving some of these important questions of nutrition. Our experiments, which have thus far been conducted with rats, have already yielded some interesting results.[29]

From a large number of experiments with many different proteins, singly and in combination, we have learned that the cause of failure in most of the previous experiments is due to an unsuitable choice of the inorganic constituents of the food. By using an inorganic salt mixture similar to that used by Röhmann, in the only approximately successful artificial feeding experiments heretofore reported, we have succeeded in keeping rats, not only in positive nitrogen balance, but in full weight and perfect health over long periods of time. Thus in our most successful experiment we have kept a rat for ten months; for the first two months on a mixture containing milk casein and wheat glutenin, and for the succeeding eight months on one containing wheat glutenin as its only protein. Many other rats have been kept in fine nutritive condition for long periods with casein or mixtures of casein with other proteins. When the conditions for such experiments are well established we expect to extend them to the many forms of food proteins with the hope of learning something more definite than is now known of the effect of differences in the constitution of the food protein on nutrition.

One of the most interesting results of our experiments has been the discovery of the fact that while a mature rat can be maintained on a food containing the isolated proteins, a young rat, on the same food, fails to make more than the slightest growth. Three young rats which weighed from 60 to 70 grams each have been fed for more than three months with the same glutenin food, which fully satisfied all of the requirements of the mature rat just mentioned. All of these have remained nearly stationary in weight, although during

[29] Carnegie Institution of Washington Publication 156 and 156 part II.

the entire period the food intake has been fully equal to that of other rats of similar weight which were in full normal growth. Many experiments with other proteins, including milk casein, have given a similar result, and we have also found that such stunted animals when transferred to a normal mixed diet, or to one containing milk powder, at once grew normally. Future investigations must show whether or not this means that a mature animal actually constructs little if any new tissue protein from its food protein, and that a growing animal, which must do so, is unable to utilize proteins of widely different constitution from its tissue proteins for this purpose. The possibility that this may be so is suggested by the interesting experiments of Aron,[30] who finds that dogs which are kept on such a restricted quantity of mixed food that they do not increase in weight during long periods grow in size at the expense of their muscular tissues, so that their skeletons are equal in weight to those of normally nourished dogs which have doubled their weight during the same time. Although we have not yet measured the different parts of our stunted rats it is evident from their appearance that their skeletons have not developed to any marked extent. They look exactly as if tissue growth had entirely ceased from the beginning of feeding with the single protein, and, if this is so, it is possible that they cannot make new tissue from the pure protein of their food. The slight growth made by some animals when thus fed can easily be attributed to the activity of intestinal bacteria which convert a small part of the food protein into other forms from which new tissues could be constructed. This possible participation of bacteria immensely complicates the conduct of such feeding experiments, and renders the interpretation of the results difficult, for even if the fæces are in some way collected so that the animal cannot eat them we have no assurance that dead bacteria may not be digested within the intestine and thereby supply the animal with substances which have been carefully excluded from the food.

[30] Aron: Biochemische Zeitschrift, 1910, xxx, p. 207.

THE NATURE OF UNIT CHARACTERS*

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AN old but ever recurring question in regard to heredity is this: Does one generation inherit any part of the experience of the previous generation? In other words, is a character acquired by one generation inherited by the next? This question, first raised in concrete form by Weismann, has been discussed *pro* and *con* for many years, but the consensus of scientific opinion at the present time favors Weismann's idea that acquired characters are not inherited. In forming a judgment on this question we should bear in mind one fundamental fact, that in the higher animals body and germ-plasm are distinct. That is, the body is distinct from the reproductive cells which it contains, and out of which the next generation is produced. Influences which affect the body have no necessary influence on the germ-cells.

Weismann some years ago demonstrated this experimentally for mutilations of the body. When the tails of mice were cut off generation after generation it was found that young of the mutilated parents had tails as long as other mice. More direct evidence of the independence of germ-plasm and body is furnished by an experiment recently performed by Dr. Phillips and myself.

A young female albino guinea-pig approaching sexual maturity (Pl. 1, Fig. 1) was deprived of her ovaries, and into her body was introduced the living ovary of a freshly killed black guinea-pig about three weeks old (Pl. 4, Fig. 17). She was later mated with an albino guinea-pig (Pl. 1, Fig. 2). By him she bore two litters of living young, and died pregnant a little over one year after the operation containing a third litter (Pl. 1, Figs. 3–8). Had she not been operated upon, her young by this male would undoubtedly have been albinos, for albino

* Delivered February 25, 1911.

guinea-pigs produce only albino young, as several investigators have clearly shown. But those young which she did bear were without exception black, which character clearly they owed to the fact that they developed from eggs produced by the ovary taken at a very immature stage from a black animal. From evidence such as this we conclude that we cannot affect the inheritance by modifications of the body of the parent, not even when the body is completely changed, since the body so far as heredity is concerned is merely a *container* of the reproductive cells. *To modify the inheritance we must modify the reproductive cells.*

But the reproductive cells are not simple; they are really dual in character, made up of equivalent parts derived from father and mother. On this matter breeding experiments throw light.

If a black guinea-pig of pure race is mated with an albino, the offspring are all black, yet contain albinism as a latent or recessive character. For if one of these black offspring is now mated with the same albino, only *half* of the offspring are black, the others being albinos. And if two of the cross-bred blacks are mated with each other, one-fourth of the young, on the average, are albinos, three-fourths being black (see Plate 2). This result we explain in the following way: The cross-bred black individual received from its black parent the character black (B), and from its white parent the character white (W). In it accordingly black and white were associated together, but only the former was manifested, the white remaining hidden by the black. But in reproduction the cross-bred black individual transmits black and white in separate cells. And since these two kinds of cells are in the long run equally numerous, it follows that the cross-bred black individuals produce both black and white offspring in proportions fairly constant.

Inheritance of this sort is called Mendelian, after Gregor Mendel, who first observed and explained it. The law governing such inheritance is called Mendel's law. Such inheritance is satisfactorily accounted for by the assumption that the repro-

ductive cells are at first dual in nature, but become simple before they can function in the production of a new individual. For this assumption we have abundant evidence furnished by the direct study of the reproductive cells with the microscope. These cells, like the cells of the body in which they are contained, show in their nuclei at cell-division a fairly constant number of bodies known as chromosomes. In the worm Ascaris there are only two of these chromosomes; in the sea-urchin Toxopneustes there are thirty-six; in mice and men, about twenty-four.

A new individual arises, in sexually produced animals, out of the union of an egg with a sperm. The sperm is relatively small but its influence equals that of the much larger egg, which fact throws light on the nature of the material basis of heredity. It suggests, namely, that this material consists largely of ferment-like bodies which initiate specific metabolic processes in a suitable medium represented by the larger portion of the egg.

In egg and sperm, before their union, the chromosome number is reduced one-half, from the double to the single condition; in Ascaris, from 2 to 1; in the sea-urchin, from 36 to 18; in the mouse and in man, from 24 to 12. These reductions occur in what is called the maturation of the sexual products.

In the male, the primitive germ-cell containing the double or 2N number of chromosomes divides up into a group of four cells each containing the single or N number of chromosomes. This comes about by a failure of the chromosomes to split (as they regularly do in ordinary cell division) at one of the two cell-divisions which produce the group of four sperm cells. A tadpole-like sperm now arises from each of the cells containing the reduced number of chromosomes.

In the maturation of the egg, reduction is likewise accomplished by two cell-divisions, in one of which the chromosomes do not split as in ordinary cell-divisions. The divisions of the egg are, however, into parts of very unequal size, only one of which is fertilized, the rest failing to develop. For example, in the marine worm, Nereis, according to Wilson, the maturation of the egg occurs simultaneously with its fertilization. The

first maturation division separates off a minute cell, known as the first polar-body, and is quickly followed by a second likewise unequal division by which a second polar-body is produced. The number of chromosomes remaining in the egg nucleus is now reduced to half that in the egg before maturation. A sperm entering the egg forms within it a second nuclear body, which, like that of the egg, contains a reduced number of chromosomes. By the union of these two nuclei a new nucleus is formed which contains the double number, and from this all cells of the new individual are directly derived. In all such cells the double chromosome number is present. Similar events take place in the maturation and fertilization of animal eggs in general.

Now, when the egg of a *black* guinea-pig is fertilized with the sperm of a *white* one, or *vice versa,* protoplasmic constituents unite which in one case are able to produce a black coat, in the other a white one. These constituents, whatever they are, evidently separate from each other and pass into different cell products when the germ-cells of the cross-bred individual ripen. It seems natural to suppose that the separation occurs at the reduction of the chromosomes from the double to the single condition. Half the sperms accordingly of the cross-bred black individual bear black, half white, none both; and the same is true of the eggs. Experiment proves conclusively that this is so. Blackness and whiteness behave in crosses like indivisible units. They may be brought together repeatedly in crosses, but always separate again at the maturation of the gametes. We call them *unit-characters.* Black is a positive unit (presence of black pigment), white its corresponding negative (absence of black pigment).

Other unit-characters are quite independent, in their inheritance, of black and white. Thus, the coat of a guinea-pig may be either rough or smooth (see Plate 3). Rough is a unit-character dominant over smooth in crosses, and among the second generation offspring derived from such a cross occur three rough individuals to one smooth one. If further the rough parent is white (Fig. 15), and the smooth one dark (Fig. 13),

then the parents differ in two unit-characters and the sequel shows that these are independent units. For although the immediate offspring are all dark and rough (Fig. 14), the next generation contains four sorts of individuals representing all possible combinations of the two alternative pairs of units, viz.:

1. Smooth dark, like one grandparent. 2. Rough white, like the other. 3. Rough dark, like the parents. 4. Smooth white, a new combination (Fig. 16).

Again, length of the hair is independent of its color or its roughness. A short-haired colored animal mated with a long-haired white one produces only short-haired colored offspring, which bred *inter se* produce in the next generation young of four sorts, viz.: 1. Short dark. 2. Long white. 3. Long dark. 4. Short white.

Recombinations in such ways can be accounted for if we suppose each different unit-character to have its basis in a different material body within the cell, perhaps in a different chromosome or part chromosome. Thus suppose hair-length to have its basis in one cell structure, hair color in another, and suppose these to be independent of each other in their fusions and segregations. Then combinations will be formed as follows:

Grandparents SD and LW
Parents S(L[1])D(W)
Grandchildren SD, SW, LD, LW.

If the individuals crossed are pure and differ in three particulars, color, length, and roughness of the coat, then their grandchildren will be of eight sorts representing all possible combinations of three independent unit-character pairs, each of which has its basis in a different material body in the cell.

A great many of the characters of animals and plants behave as simple units in heredity, yielding a 3:1 ratio of dominant to recessive individuals in the second generation from the cross. I have shown that this is true of certain hair characters in guinea-pigs, viz.: blackness, roughness, and length of the hair.

[1] Characters in parenthesis, recessive, not visible.

We have no idea how numerous such characters are until they happen to be lost in one individual or another. Then a new variation, a sport or mutation, is observed. It is by this means, accidental loss of simple unit-characters, that the great color variation of domesticated animals has arisen.

We should naturally consider the color character of a wild mouse, rat, or rabbit, to be very simple, for we observe such animals to breed very true to color, but the behavior of the wild type in crosses shows it in reality to be very complex and to be the result of the simultaneous presence of some half-dozen or more wholly independent unit-characters. New color varieties have arisen by loss or modification of one or more of these unit characters. For example, the wild house-mouse, by simple loss of three independent factors, has given rise to seven additional varieties known among fancy mice.

The gray fur contains black, brown, and yellow pigments disposed in a definite pattern in the individual hairs. Loss of this pattern alone produces black. Loss of black produces cinnamon; loss of both produces brown. Loss of the power to produce color, that is loss of some general color factor, produces an albino, whose breeding capacity will vary with the number of other factors which it retains. Several other color factors occur in mice, the loss of which has produced new series of color varieties, but these will suffice to show the process by which new varieties arise through loss of unit-characters.

Simple unit-characters are not confined to the superficial parts of an animal, as, for example, to the fur. We know variations of this sort probably better than any other sort, simply because they are most easily observed.

Loss of horns in cattle behaves as a dominant unit-character; likewise in man a shortened condition of the skeleton, producing two-jointed instead of three-jointed fingers, behaves as a simple dominant unit-character. A curious affection of the nervous system producing the waltzing condition of Japanese mice, behaves as a recessive unit-character in crosses with the normal condition.

Is *all* inheritance unit-character inheritance? This ques-

tion we cannot at present answer fully, but many facts indicate that it is. A large class of seemingly unconformable cases which presented the greatest obstacle to such a view has recently been brought into line with the unit-character hypothesis. I refer to cases of blending inheritance, in which the offspring are intermediate between the parents, and this intermediate condition persists into the next generation.

Size and skeletal proportions are inherited apparently in this fashion. It is possible, however, that even in such cases unit-character segregations may really occur, though their presence is obscured because dominance does not occur. For in plants such size segregations have been observed recently by my colleague, Dr. East, and by others.

A single illustration will suffice. When varieties of maize (or Indian corn) are crossed which differ in size of ear, the hybrid plants bear ears of intermediate size, but not more variable than the more variable parent. The second generation offspring, however, are extremely variable, ranging in size from that of the smaller parent variety to that of the larger.

The peculiarity of what we have called blending inheritance lies partly in the entire absence of dominance. In blending inheritance a unit-character represented *once* in the fertilized egg has only half as much effect as one represented twice. In color inheritance, usually, but not always, a single dose of a unit-character is as effective as a double dose in causing the development of the character. In size inheritance, however, the single and double doses probably produce very different effects.

A further apparent difficulty encountered in interpreting blending inheritance as unit-character inheritance lies in the multiplicity of the units involved, so that segregations do not occur into a few discontinuous size classes but into classes so numerous and differing so little from each other that it is very difficult to distinguish them.

Suppose that in crosses of black and white guinea-pigs black were represented by *two* unit-characters B and B′, instead of by one, residing perhaps in different chromosomes, and that either

one of these could by itself produce black color, then a larger proportion than three-fourths of the second generation offspring would be black, viz., fifteen-sixteenths. If, further, the presence of a larger number of factors for black produced *more* black pigment in the fur than a smaller number produced, then we should have gradations of blackness among the second generation offspring as follows: 4, 3, 2, 1, 0.

Add a third factor for black in the supposed cross, located perhaps in a third chromosome, and the pure whites would be reduced to 1 to 64 of the second generation offspring, while the different gradations or intensities of blackness would become 6, 5, 4, 3, 2, 1, 0. The occasional white individual would now differ so little from the lightest black one that the two might often be confused, and there would seem to exist all intermediate stages between pure white and pure black, without entire segregation into either. By selecting for the lightest or the darkest condition within a mixed race of such second-generation offspring one would obtain with each selection a larger proportion of extremely dark or extremely light individuals, until a pure race was obtained. Further, if the black character should become attached to additional material bodies in the cell (chromosomes or the like) so that it would be represented by additional units, then the occurrence of light colored progeny would become still rarer, and deeper intensities of blackness than before existed would now occur. Thus selection would become a means for the apparent modification of a character really dependent upon the inheritance of unchanging units. Now this is perhaps what occurs when one seeks to modify size by selection.

There are strong reasons for believing that Mendelizing characters can be modified by selection, though this idea is vigorously combated by many Mendelians, notably by Johannsen. In his view selection can do nothing but sort out variations already existing in a race. I prefer to think with Darwin that selection can do more than this, that it can heap up quantitative variations until they reach a sum total otherwise unattainable, and that it thus becomes creative. I will describe briefly certain

experiences of my own which support this idea. In several cases I have observed characters at first feebly manifested gradually improve under selection until they became established racial traits.

Thus in guinea-pigs the hind-foot commonly bears three toes (Plate 4, Fig. 19 *B*). But several years ago I observed an individual which had an imperfectly developed fourth toe similar to that shown at *D*, Fig. 19. From the descendants of this animal obtained by inbreeding and selection was formed a race having well-developed fourth toes on both hind-feet.

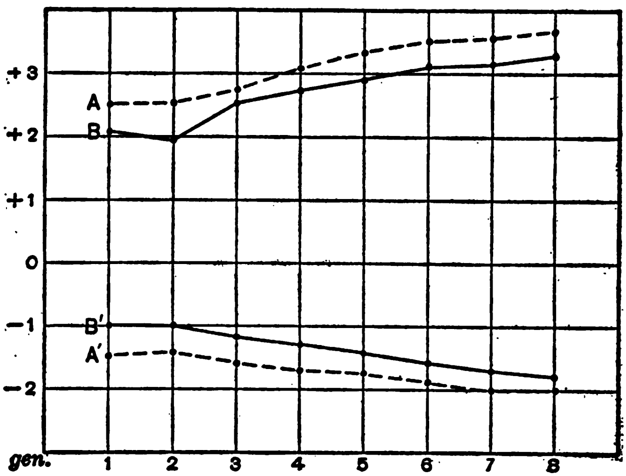

FIG. A.—Chart showing effects of selection in eight successive generations upon the color-pattern of hooded rats. *A*, average condition of the selected parents in the *plus* series; *B*, average condition of their offspring. *A*¹, average condition of the selected parents in the *minus* series; *B*¹, average condition of their offspring.

The extra toe made its appearance, poorly developed, on the left foot only. About 6 per cent. of the offspring of this animal by normal unrelated mothers were polydactylous, but among his offspring were some with better developed fourth toes than the father possessed. Such individuals were selected throughout five successive generations, at the end of which time a good four-toed race had been established. It was found in general that those animals which had best-developed fourth toes trans-

mitted the character most strongly in crosses with unrelated normal animals. The percentage of polydactylous individuals produced in such crosses varied all the way from 0 to 100 per cent. By selection this percentage was increased, as was also the degree of development of the fourth toe in crosses.

Another character which made its appearance among our guinea-pigs, at first feebly expressed, was a silvering of the colored fur, due to interspersing of white hairs with the colored ones. The first individuals observed to have this character bore white hairs on the under surface of the body only. By inbreeding, a homozygous strain of the silvered animals was soon obtained, one in which all the offspring were silvered to a greater or less extent. Selection was now directed toward two ends,—(1) to secure animals which were free from spots of red or white, a condition which was present in the original stock, and (2) to secure extensive and uniform silvering on a black background. In both these objects good progress has been made. We have animals which are silvered all over the body except on a part of the head, and the percentage of such well-silvered individuals is relatively high.

But the most extensive selection experiment which I have personally observed is one in which I have been assisted by Dr. John C. Phillips (see Fig. 18). Selection in this case has been directed toward a modification of the color pattern of hooded rats,—a pattern which is known to behave as a recessive Mendelian character in crosses with either the self (totally pigmented) condition or the so-called Irish (white-bellied) condition found in some other rats. The extreme range of variation among our hooded rats at the outset of this experiment is indicated by the grades —2 and +3 of Fig. 18. Selection was now made of the extreme variates in either direction and these were bred separately. Two series of animals were thus established,—one of narrow striped animals, *minus* series; the other of wide striped, *plus* series. In each generation the most extreme individuals were selected as parents; in the narrow series, those with narrowest stripe; in the wide series, those with widest stripe.

The result of the selection is shown graphically in text-figure (compare Table I). The offspring in the narrow series became with each generation narrower; those in the wide series became with each generation wider, with a single exception. In generation two the wide stock was enlarged by the addition of a new strain of animals. This caused a temporary falling off in the average grade of the young, the two series overlapping for that generation. No new stock was at any other time introduced in

TABLE I

RESULTS OF SELECTION FOR MODIFICATION OF THE COLOR-PATTERN OF HOODED RATS

	Generation.	Average Grade, Parents.	Average Grade, Offspring.	Number of Offspring.
Plus series.	1	2.50	2.05	150
	2	2.51	1.92	471
	3	2.73	2.51	341
	4	3.09	2.72	444
	5	3.33	2.90	610
	6	3.51	3.09	834
	7	3.53	3.14	874
	8	3.65	3.30	91
				3815
Minus series.	1	1.46	1.00	55
	2	1.41	1.07	132
	3	1.56	1.18	195
	4	1.69	1.28	329
	5	1.73	1.41	701
	6	1.86	1.56	1252
	7	2.00	1.70	1544
	8	2.03	1.78	713
				4921

either series, the two remaining distinct at all times except in generation two. It will be observed that a change in the average grade of the parents is attended by a corresponding change in the average grade of the offspring. The amount of variability of the offspring is not materially affected by the selection,

but the average about which variation occurs is steadily changed, as are also the limits of the range of variation.

The interesting feature of this experiment is the production, as a result of selection, of wholly new grades; in the narrow series, of animals having less pigment than any known type other than the albino; in the wide series, of animals so extensively pigmented that they would readily pass for the "Irish type," which has white on the belly only, but which is known to be in crosses a Mendelian alternative to the hooded type. By selection we have practically obliterated the gap which originally separated these types, though selected animals still give regression toward the respective types from which they came. But this regression grows less with each successive selection and ultimately should vanish, if the story told by these statistics is to be trusted. As yet there is no indication that a limit to the effects of selection has been reached.

From the evidence in hand we conclude that Darwin was right in assigning great importance to selection in evolution; that progress results not merely from sorting out particular combinations of large and striking unit-characters, but also from the selection of slight differences in the potentiality of gametes representing the same unit-character combinations.

Accordingly we conclude that unit-characters are not unchangeable. They can be modified, and these modifications come about in more than a single way. Occasionally a unit-character is lost altogether or profoundly modified at a single step. This is mutation. But more frequent and more important probably are slight, scarcely noticeable modifications of unit-characters that afford a basis for a slow alteration of the race by selection. Mutation, then, is true, but it is a half-truth; selection is the other and equally important half of the truth of evolution as Darwin saw it and as we see it.

CALCIFICATION AND OSSIFICATION*

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IT is the merit of Jacques Loeb to have shown us the necessity of simplifying our methods of attacking biologic problems. Instead of investigating the effects of complex substances on still more complex living organisms, he took up the simplest things imaginable, the familiar inorganic salts, and for his living subject the least complex complete organism possible—the single cell, *sans* blood, *sans* nerves, *sans* soul, *sans* everything. We pathologists cannot so readily reduce our subjects of investigation to such simple terms, but are forced too often, by the nature of our problems, to the opposite extreme, with such unsatisfactory results as might be expected. The situation of a pathologist, trying to investigate the action of a bacterial toxin of absolutely unknown composition on the equally unknown compounds of a complex mammalian organism, reminds us pathetically of the grotesque figure in "Confessio Medici," of the blind man searching in a dark room for a black hat which is not in the room. To be sure, in our gropings we have occasionally blundered into some great truth, but I fear that when our eyes are opened and the daylight comes, we shall be shocked at the wreckage our blind efforts have caused. For example, is it not possible that, stumbling about in the darkness, we have buried the simple key to the mystery of immunity beneath a mass of false data and distorted facts?

Calcification, dry and unpromising a problem as it may seem to be, possesses at least one of the cardinal virtues on which Loeb has insisted: The participants at one end of our reactions are of known and simple nature. Calcium salts we can isolate, estimate, and investigate to our hearts' content, and with all the accuracy which modern chemical methods afford us. And,

* Delivered March 25, 1911.

indeed, the other end of the reaction seems to be somewhat simpler than the biological reactions of living cells, for in pathological calcification the organic structures are commonly dead, inert, chemically inactive; and even in ossification a relative chemical inactivity seems to characterize the calcifying structure. A pathologist working along chemical lines, therefore, has the right to feel that in the field of calcification he has a favorable place in which to try his feeble flights with the best hope of success.

The accumulation and deposition of insoluble calcium salts by living organisms is so universal a process, exhibited by even the simplest protozoa, that in the investigation of the problems of calcification and ossification one is carried far afield into the territory of the zoölogist, the biologist, and the plant physiologist. On the chemical side we find the newer developments of colloid chemistry coming to our assistance with many suggestive disclosures, and so our problem, which at first may seem somewhat limited, broadens out into a most interesting one. Furthermore, the existence of such diseases as rickets, osteomalacia, and osteoporosis, and questions concerning repair and regeneration of bone, make the discussion of more than merely academic interest.

Calcification, as observed in the formation of shells by mollusks and crustacea, seems to be a comparatively simple matter, and if the commonly accepted interpretation of the chemical processes is correct, we can find here little to help us in our study of physiological and pathological calcification in the mammals. The essential discrepancy is that with the latter the calcium salts are brought to the place of deposition in the blood of the animal, while with the marine invertebrates the calcium is apparently provided by the surrounding fluid. The hypothesis of Murray and Irvine,[1] which Von Fürth[2] seems to

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[1] Murray and Irvine: Coral Reefs and Other Carbonate of Lime Formations in Modern Seas, Proc. Royal Soc., Edinburgh, 1889, xvii, 79.

[2] Von Fürth: Vergleichende chemische Physiologie der niederen Tiere, Jena, 1903, p. 578.

consider the most adequate explanation of the facts, is that the calcium of the molluscan shells and of coral reefs is provided by the sea-water, in which it exists dissolved in the form of the relatively soluble sulphate, and that it is precipitated as calcium carbonate by the ammonium carbonate which is formed in the metabolism of the animals. This carbonate is excreted through the integument, where the deposition takes place according to the following equation:

$$CaSO_4 + (NH_4)_2CO_3 = CaCO_3 + (NH_4)_2SO_4$$

Another important difference is that in all these lower forms the calcium deposits consist chiefly of carbonate, with but little phosphate, whereas in the mammals and in most other vertebrates the calcium deposits are chiefly phosphate with relatively little carbonate. An important exception to this last statement is furnished by the shells of birds' eggs, the calcium in these being almost all carbonate,[3] and this in spite of the fact that the bones of birds closely resemble the bones of mammals in composition, and consist chiefly of phosphate with about one-sixth as much carbonate of calcium (Hiller,[4] Weiske[5]). A particularly remarkable feature of the formation of egg-shells is the constancy of composition, for if a hen is deprived of calcium until she begins to lay eggs with very little inorganic matter in the shell membranes, on the addition of silicate, phosphate, nitrate, or sulphate of calcium to the food, she

[3] Shells of hens' eggs contain approximately 97 per cent. $CaCO_3$, 1 per cent. $Ca_3(PO_4)_2$, including some small quantities of magnesium phosphate and carbonate, and also 2 per cent. of organic matter. (Bronn: Klassen und Ordung des Thierreiches, 1891, vi, 875.) This carbonate probably exists in the form of calcit. (Kelly, Agnes: Beiträge zur mineralogischer Kentniss der Kalkansscheidung im Tierreiche, Jenaische Ztschr. f. Naturwissensch., 1901, new series, xxviii, 429.)

[4] Hiller: Vergleichende Knochenuntersuchungen am Skelett eines Vogels, Landwirts. Versuchsstat., 1885, xxxi, 319.

[5] Weiske: Untersuchungen über Qualität und Quantität der Vogel-Knochen und Federn in verschiedenen Altersstadien, Landwirts. Versuchsstat., 1889, xxxvi, 81.

will begin, in a few days, to lay eggs with shells of the usual thickness and containing calcium carbonate in normal proportion (Murray and Irvine). Concerning the manner and means by which the egg-shells are formed, we know very little, beyond that in the hen the egg usually remains about twenty hours in that part of the generative tract where the shell is formed. The interesting experiments of Pearl and Surface[6] show that the shell-glands secrete the calcium in response to mechanical stimulation, for when they anastomosed the intestine to the oviduct the fæces which passed over the "shell-glands" were covered with a calcareous shell. This observation recalls Leo Loeb's demonstration that the formation of the maternal placenta in guinea-pigs takes place when a mechanical stimulus acts upon a uterine mucosa which has been sensitized by a hormone from the corpus luteum.

How the daily deposition of 4 or 5 Gm. of calcium carbonate is accomplished by laying hens has not been explained. As hen's blood contains but 0.007 to 0.020 per cent. of calcium oxide, all the calcium of about 15,000 to 30,000 c.c. of blood must be secreted by the shell-glands to form an average shell, which contains 2 or 3 Gm. of calcium oxide. One may imagine that the calcium is carried through the walls of the shell-glands in the form of the soluble bicarbonate, the carbonate being precipitated either by a neutralization process, or by simple escape of the excessive carbon dioxide. The chemistry of shell formation by birds seems to have received little investigation, as yet, although it is an interesting problem; but its solution probably will give us little information as to how calcium phosphate is deposited in mammalian tissues.

THE RELATION OF CALCIFICATION AND OSSIFICATION

With the double topic of calcification and ossification before us, we may properly begin by ascertaining whether we are here concerned with two separate processes, or with two manifestations of a single process. The essential differences between

[6] Pearl and Surface: The Nature of the Stimulus which Causes a Shell to be Formed on a Bird's Egg, Science, 1909, xxix, 428.

ossification and calcification seem to be chiefly morphological. In calcification we have deposited in dead tissues, or in tissues of low vitality, a considerable quantity of inorganic calcium salts, which appear at first in granular form, although later there may be more or less fusion and resulting areas of homogeneity. Within such deposits there are usually no living cells, and, so far as we know, no further changes take place in the calcified area unless it be absorption or addition of more calcium salts. We have no information as to whether resorption of this calcium can take place to supply a deficit in the diet or the unusual demands of lactation and pregnancy, but we do know that it is not necessarily permanent, for the experimentally produced deposits of calcium in the kidney of the rabbit may be reabsorbed (Von Werra [7]) within a few weeks, and even if such deposits have undergone secondary ossification they may disappear within a year (Maximow [8]). In normal ossification, however, the homogeneous calcium deposits are closely related to living cells, which not only determine the form of the deposits, but which also are able to dissolve the insoluble salts or to cause their deposition as may be needed, thus rendering the inorganic salts of bone the reserve supply of a tissue of active metabolism, entirely comparable to deposits of glycogen or of fat, and perhaps quite as important in view of the necessity of maintaining strict neutrality of the blood, a vitally important process in which the bone salts are of the utmost value.

Beyond this, however, there seem to be no differences between normal ossification and pathological calcification. Even morphologically there are many points of resemblance. In each case the insoluble salts are laid down in a matrix especially prepared to receive them; in bone formation the homogeneous acellular matrix of the cartilage; in calcification some acellular necrotic tissue, or, more especially, homogeneous elastic fibres

[7] Werra, von: Ueber die Folgen des vorübergehenden und dauernden Verschlusses der Nierenarterie, Virchow's Arch. f. path. Anat., 1882, lxxxviii, 197.

[8] Maximow: Ueber experimentelle Erzegung von Knochenmarkgewebe, Anat. Anz., 1906, xxviii, 609.

or hyaline degenerated connective tissue, each of these latter bearing marked structural resemblance to the hyaline matrix of the cartilage or osteoid tissue. If we dissolve out the salts with acids we find remaining alike in bone and calcified areas an insoluble ground substance of homogeneous organic material, usually showing an affinity for basic dyes; however, the proportion of salts and stroma is less constant in pathological calcification than it is in adult bone. Finally when the inorganic salts are first deposited in normal ossification they are in a finely granular form (Pacchioni [9]) even although they later appear to be homogeneous, so that even this distinction between calcification and ossification is not absolute. Chemically the resemblance is even closer, for with few exceptions the proportion of the different inorganic salts in all sorts of areas of pathological calcific deposits has been found quite the same as that characteristic of normal bone. I grant that in the literature there may be found a few analyses of pathological materials indicating that the calcium salts present were not in a proportion similar to that of the inorganic substance of normal bone, which in the mammals is invariably from 85 to 90 per cent. of calcium phosphate and from 10 to 15 per cent. calcium carbonate, but there is every reason to believe that these atypical results are to be ascribed to improper methods of analysis. Nothing can be more misleading than to analyze an ash for bases and acids, and then to give the resulting figures as indicating the composition of the inorganic elements present in the tissues during life. In the case of bone-ash the results for the carbonate will inevitably be incorrect, for during the burning there develops a great quantity of carbon dioxide, more or less of which will unite with the bases present; with subsequent heating, however, all the carbon dioxide can be driven off, leaving only the oxides of the metals. In a test experiment, 2 Gm. of pure gelatine and 2 Gm. calcium phosphate [$Ca_3(PO_4)_2$] were mixed by means of a little hot water, dried, and fused over a Bunsen burner until only a little carbon

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[9] Pacchioni: Untersuchungen über die normale Ossification des Knorpels, Jahrb. f. Kinderh., 1902, lvi, 327.

was left, as shown by the gray color of the ash. Analysis showed the presence in this ash of 0.0356 Gm. carbon dioxide, which indicates the presence of 0.080 Gm. calcium carbonate ($CaCO_3$); therefore, about 4 per cent. of the calcium of the phosphate had become united as carbonate. A repetition of this experiment, carrying the fusion until there was practically no free carbon left, yielded 0.026 Gm. carbon dioxide, or 0.059 Gm. calcium carbonate.

The danger of error from addition or loss of carbon dioxide in ashing, although generally recognized in bone analyses, seems to have been overlooked in several of the reports of analyses of pathological calcific deposits which speak of the amount of carbon dioxide in the ash. That the phosphoric acid determinations in ash are also unreliable, seems not to have been considered, even in most of the recorded analyses of bone. We have, however, in bone tissue, a greater or less proportion of nucleoproteins and lecithin, especially when the marrow is included, from which phosphoric acid is freed on heating, and which will unite with the bases, especially the carbonates.[10] For example, in a test analysis, a mixture containing (1) 2 Gm. of $CaCO_3$; (2) 2 Gm. of dog spleen from which all phosphorus soluble in water, ether, or boiling alcohol had been extracted; and (3) 0.5 Gm. lecithin, was fused until white, washed thoroughly with water to remove soluble phosphates, and analyzed. No less than 0.0635 Gm. of insoluble P_2O_5 was found, corresponding to 0.1344 Gm. of $Ca_3(PO_4)_2$, which had undoubtedly been formed from the organically bound P_2O_5 during the fusion, changing 6.6 per cent. of the calcium of the carbonate into phosphate.

There have been made in my laboratory by R. L. Benson, Conrad Jacobson, and myself, many analyses of normal and

[10] The method used by Gabriel, decomposing the bone with an alkaline solution of glycerin, offers the same opportunity of converting organic phosphorus compounds into calcium phosphate, and in fact gives the same proportion of calcium phosphate as the ashing method. It is an interesting fact, emphasized by Aron (Stutzgewebe und Integumente der Wirbeltiere, Handbuch der Biochemie, 1908, ii, 178), that both methods indicate the presence of sodium and potassium held in the bone ash in an insoluble form.

pathological calcified materials, both natural and experimental, by a method devised to exclude these sources of error, and without exception, whether the material was early or late calcification, large or small in amount, natural or experimental, human, bovine, or rabbit, we have always obtained results showing that in pathological deposits the calcium salts are always present in the ratio of phosphate and carbonate which is, within certain limits, characteristic of normal bone. Of the analyses in the literature, a large proportion show a similar ratio of carbonate and phosphate. Where the results are different from this they usually can be explained by the use of the ashing method in the presence of a large amount of organic material.

PATHOLOGICAL CALCIFICATION

Perhaps the most striking evidence of the relation of calcification to ossification is the frequency with which we find an area of pathological calcification of some dead tissue undergoing a metamorphosis into true bone. Surely nothing can be more remarkable, more spectacular, indeed, than that a human eye may come to be lined with a shell of true living bone, perhaps containing marrow, as a sequel to the deposition of calcium salts in dead material left unabsorbed after a suppuration within the eye. Neither is this an unusual, isolated observation, for we find it to be a rule that when areas of calcified pathological tissues remain long enough in the body, ossification will take place in a certain proportion of cases, irrespective of any proximity or relation to bone tissue. This is particularly true of the eye, the ophthalmologists having reported many cases of ossification of calcific deposits within the eyeball, and Poscharissky [11] found that of twenty-nine such calcified areas which he examined, true bone could be found in all but four. Calcified nodules from the lungs of twenty-eight persons were examined by the same author, and in seventeen of the bodies (60 per cent.) bone was found. Bone-marrow, or a tissue

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[11] Poscharissky: Ueber heteroplastische Knochenbildung, Beitr. z. path. Anat. u. z. Allg. Path. (Ziegler's), 1905, xxxviii, 135.

resembling it, was found in three of fourteen calcified nodules from heart-valves, and bone was present in four of thirty-one calcified aortic plaques. Calcified nodules in the liver and in the mesenteric glands are also often ossified, and in fact there is hardly a tissue in the animal body in which transformation of calcified into ossified tissue has not been observed (Harvey[12]).

The process by which these inert dead calcified areas are converted into living bone tissue is entirely analogous to the normal formation of bone in endochondral ossification. The calcified material simply takes the place of the primordial cartilage, vascular granulation tissue eroding it, the cells of the granulation tissue undergoing a differentiation into osteoblasts which constitute an osteogenetic layer and form the new bone. When there is no preliminary calcification of a necrotic tissue we get no subsequent ossification, and it seems that the *calcium salts exert a specific influence on the connective tissue cells which causes them to take on active growth, and to undergo a metaplasia, not only into osteoblasts and bone corpuscles, but apparently even into marrow cells with hæmatogenetic function,* since, according to Bunting [13] and others, the evidence indicates that the marrow tissue which so commonly accompanies pathological ossification [14] is derived from the proliferated connective tissue cells.[15] According to Poscharissky a further point of resemblance between normal and pathological ossification is that in senile cartilage which is undergoing or is about to undergo ossification, the characteristic microchemical tests for amyloid can be obtained, and the same reactions are given by the decalci-

[12] Harvey: Experimental Bone Formation in Arteries, Jour. Med. Research, 1907, xvii, 25.

[13] Bunting: Formation of True Bone with Cellular, Red Marrow in a Sclerotic Aorta, Jour. Exper. Med., 1908, viii, 365.

[14] Poscharissky (see Note 11) states that marrow tissue is *always* present when bone is formed in calcified tissues, and that in 10 per cent. of his cases there was marrow without true bone, but it is possible that he is too liberal in his interpretation of the histological criteria of marrow.

[15] Maximow (see Note 8) ascribes the marrow formation to metaplasia of lymphocytes from the blood.

fied ground substance of ossifying calcified areas.[16] It is nothing less than remarkable how rapidly ossification can occur in areas of pathological calcification, for Liek[17] was able to obtain bone formation constantly in the pelvis of the rabbit's kidney within sixteen or twenty days after ligating the renal artery, provided that he wrapped the kidney with omentum to secure a free collateral circulation; without this collateral circulation it requires about three months for ossification to appear. These and other experiments by the same author show that the amount of blood-supply is an important factor in determining pathological ossification; with too free a circulation there is no calcification and no ossification, with too little circulation necrosis is followed by slow calcification and either late or no ossification.

The essential part played by the calcium salts in stimulating osteogenesis is further demonstrated by Barth's[18] experiments on the healing of bone defects by implantation of dead and living bone. He found that living bone thus implanted always dies, and then the dead bone is replaced by a process of substitution, new layers of osteoid tissue invading and replacing layer by layer the dead bone. If the bone is dead and sterile when implanted, or if ashed bone is used, the results are quite the same. Calcium sulphate placed in bone cavities also favors rapid ossification.[19] If, however, decalcified bone is similarly implanted it is quickly absorbed, and is replaced by fibrous

[16] The only attempt with which I am familiar, to corroborate this interesting observation was made by Buerger and Oppenheimer (Bone Formation in Sclerotic Arteries, Jour. Exper. Med., 1908, x, 354) with negative results in the one specimen of ossifying arteries which they examined. It may be recalled that Wichmann [Die Amyloiderkrankrung, Beitr. z. path. Anat. u. z. allg. Path. (Ziegler's), 1893, xiii, 487)] also throws doubt on many of the earlier statements concerning the presence of amyloid in cartilage and bone.

[17] Liek: Heteroplastische Knochenbildung in Nieren, Arch. f. klin. Chir., 1908, lxxxv, 118.

[18] Barth: Histologische Untersuchungen über Knochenimplantationen, Beitr. z. path. Anat. u. z. allg. Path. (Ziegler's), 1895, xvii, 65.

[19] Barth: Ueber künstliche Erzegung von Knochengewebe und über die Ziele der Osteoplastik, Berl. klin. Wchnschr., 1896, xxxiii, 8.

tissue, without ossification except such growth of bone as may invade the scar tissue from the living bone tissue about the margins. Stoeltzner[20] has also indicated the importance of calcium in natural ossification, not only as forming a part of the bone, but also in stimulating osteogenic tissue to form bone.

The observations cited above indicate to us the specific osteogenetic influence exerted by deposits of calcium salts, alike whether in dead tissue or in cartilage which is to be ossified. But how is this influence exerted? It is hard to imagine that it is chemical, since the calcium salts concerned are most insoluble, and it does not seem probable that the fluids bathing them will contain appreciably more calcium than the normal body fluids, which seem to be pretty nearly saturated with calcium salts. Possibly it is a tactile stimulus—if so we might have ossification induced by rough mineral deposits other than calcium, but such a thing has never been described. In any case, in order to have ossification of calcific deposits, certain conditions of relationship between calcium salts, fibrous tissues and blood-supply evidently must be very exactly met, as shown by Liek's experiments with the rabbit's kidney, and by his failure to produce ossification by the implantation of pieces of decalcified bone and masses of calcium phosphate or carbonate into the peritoneal cavity and soft tissues of rabbits.[21] Morpurgo and Martini[22] also obtained negative results in similar experiments. On the other hand, even in extreme old age the senile connective tissues are still able, under suitable conditions, to undergo active osteogenetic metamorphosis (Bunting).

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[20] Stoeltzner, W.: Die zweifache Bedeutung des Calciums für das Knochenwachstum, Arch. f. d. ges. Physiol. (Pflüger's), 1908, cxxii, 599.

[21] Barth (see Note 18) states that he found some isolated islands of young bone in a piece of bone ash implanted into the peritoneal cavity of a cat six weeks previously, which Liek believes due to a misinterpretation of the histological findings.

[22] Morpurgo and Martini: Atti d. R. Accad. d. fisiocrit. di Siena, 1898, x, quoted by Sacardotti and Frattin: Virchow's Arch. f. path. Anat., 1902, clxviii, 431.

THE PROBLEMS OF CALCIFICATION

All the above facts, when taken together, leave little ground for the belief that there is any essential dissimilarity between the processes of ossification and calcification. In either case histological elements of similar structure are infiltrated with inorganic salts of identical composition, and even if at first the calcific area is dead, inert, and of pathological origin, it may later by gradual change be transformed into true living bone. Therefore it is permissible for us to consider both processes together, and the evidence afforded by either can be applied to the other with but slight modification and few reservations. We may summarize the problems awaiting solution under the following heads:

1. Why is calcium deposited in the tissues at all?
2. Why is it deposited in some tissues and not in others?
3. How is the calcium carried in the blood?
4. How is it held in the tissues where deposited?
5. Why is the composition of calcified deposits so constant, qualitatively and quantitatively?
6. What are the causes of rickets and osteomalacia?

In the first problem we must consider in the beginning that in mammals only one normal tissue is the site of calcific infiltration, the developing bone, while *any* tissue may become calcified provided that its vitality is reduced sufficiently and that it remains long unabsorbed. Even such highly specialized structures as the ganglion cells of the brain may become calcified so completely that there results a perfect cast of the original cell, dendrites, axis cylinder and all. Furthermore, not all cartilages calcify, and indeed only certain portions of those cartilages which are eventually to become entirely ossified are calcified at first. Why is it that the ribs calcify up to a certain definite line, leaving the costal cartilage uncalcified for a long space of years, only eventually to undergo in old age a final senile calcification and ossification? Who do only certain particular spots in the great mass of fœtal cartilage become calcified, one by one and in a definite order, until finally only a

narrow margin of cartilage is left about each to form the joint surfaces? In all these cases the process seems to follow a definite order, with an early deposition of calcium salts; this is followed by an increased vascularity at these places, the newly-formed vessels and the cells which accompany them forcing their way into and partly absorbing the calcified cartilage; a new structure is now laid down in place of the resorbed cartilage, containing the invading cells in the form of osteoblasts and bone corpuscles; this new tissue is then infiltrated with calcium salts and we have true bone. This series of processes, it will be seen, is exactly the same as occurs in the ossification of pathological calcified areas, and in each case it is the deposition of calcium which is the primary step. Therefore the problem of ossification is, after all, the problem of calcification of tissues, normal or pathological.

THE CHEMICAL FIXATION THEORY

To account for the primary deposition of calcium salts (for the magnesium and other bases are relatively so insignificant in amount that we can disregard them for the present) many hypotheses have been advanced, and none is simpler or older than the idea of a precipitation of insoluble salts of calcium because of a chemical reaction, certain acid radicals formed within the tissues combining with and precipitating the calcium contained in the blood and tissue fluids. To this idea no better name can be given than that used by the Germans, the *Kalkfänger* hypothesis.

As most of the calcium of bones is phosphate, the first idea that occurs is that phosphoric acid is the *Kalkfänger*, and we have no difficulty in finding possible sources of phosphoric acid. In ossification it might come from autolysis of the nuclei of the cartilage cells (Grandis and Mainini [23]), or these cells might secrete soluble phosphates; in calcification P_2O_5 might come from the autolysis of the nucleoproteins and lecithin of the dead tissues. Such an explanation, however, has failed of

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[23] Grandis and Mainini: Arch. ital. de biol., 1900, i, 73.

proof. In calcified necrotic areas there is far more inorganic phosphoric acid than could possibly be derived from the phosphorus which was originally held there in organic combination; so, too, in ossification, the amount of nuclein phosphorus present in cartilage is much too small to account for the phosphates present in the bone, and the traces of soluble inorganic phosphorus present in ossifying cartilage are not greater in amount than in other, non-calcifying tissues.[24] Furthermore, I have found by experiment that dead sterile pieces of tissue rich in nucleoprotein (thymus and spleen) do not take up calcium more rapidly than do similar pieces of tissue poor in phosporus compounds (muscle [25]). One might, of course, imagine an active secretory formation of phosphoric acid by the osteoblasts, but such a process has not been demonstrated, and we shall later give evidence which apparently excludes this possibility.

Another way in which we might account for the phosphate in the event phosphoric acid is not the true *Kalkfänger,* is by double decomposition; any precipitated calcium salts of whatever sort present in the tissues being converted into the phosphate, because this is one of the most insoluble of calcium salts, and, according to the law of double decomposition, the least soluble salt is formed when solutions of two salts are mixed. It is unquestionably true that other calcium salts within the tissues do become replaced to at least some extent by phosphate, as we have found true for calcium sulphate [26] implanted into the tissues of rabbits. In view of the fact that calcium sulphate is a salt of calcium which is only slightly soluble, and that cartilage is characterized by containing a compound of sulphuric acid with chondrosin (chondroitin-sulphuric acid) this source of a possible *Kalkfänger* is at once evident. But since the calcium

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[24] Wells and Benson: Studies on Calcification and Ossification II, Jour. Med. Research, 1907, xii, 15.

[25] Wells: Pathological Calcification, Jour. Med. Research, 1906, xiv, 491.

[26] Wells and Mitchell: Studies in Calcification and Ossification, III, Jour. Med. Research, 1906, xxii, 501.

salt of chondroitin sulphuric acid is very soluble, this compound itself cannot precipitate calcium, and the total amount of SO_4 present in cartilage is far too small to account for any appreciable precipitate of calcium sulphate—indeed it would require quite a considerable quantity of calcium and SO_4 present together at one time in the tissues to produce a precipitate, in view of the relatively considerable solubility of $CaSO_4$ (2.73 Gm. per litre in water at 18° C., Kohlrausch and Rosa [27]), and numerous analyses have failed to show the presence of an appreciable amount of $CaSO_4$ in calcifying tissues at any stage of the process.[25]

Carbonic acid certainly cannot be looked on as a precipitant, since the more CO_2 present the more soluble are the calcium salts, which readily form the soluble bicarbonate; indeed it is probable that solution of bone takes place through this reaction.

CALCIUM SOAPS

The available inorganic acids being exhausted by the foregoing list, we must fall back on a possible organic *Kalkfänger,* and here several well-known facts at once call our attention to the fatty acids. In the first place, we know that calcium soaps do at times form in pathological processes, as seen especially in areas of fat necrosis where the formation of calcium soaps is an almost constant process. Indeed, necrotic areas in fat tissue commonly form calcium soaps, calcification of a lipoma with the presence of calcium soaps having been described as long ago as 1851 by Fürstenberg,[28] while Jaeckle [29] found 29.5 per cent. of the calcium in a calcified lipoma combined with fatty acids. Secondly, areas of pathological tissues which calcify are commonly areas in which fatty degeneration has taken place, *e.g.*, caseous tubercles, atheromatous vessels, old infarcts, etc.

It is easy to imagine calcium soaps formed in this way be-

[27] Kohlrausch and Rosa: Ztschr. f. physik. Chem., 1893, xii, 234.

[28] Fürstenberg: Magazin f. Tierheilk., 1851, xvii, 1; quoted by Hofmeister. See Note 30.

[29] Jaeckle: Ueber die Zusammensetzung des menschlichen Fettes, Ztschr. f. physiol. Chem., 1902, xxxvi, 53.

coming slowly transformed *in situ* into calcium phosphate and carbonate, by double decomposition in the presence of soluble phosphates and carbonate formed in the degenerating tissues or brought from the blood, and the hypothesis is most plausible. We must, indeed, admit that calcification by soap formation does occur, as witness the calcification of foci of fat necrosis and lipomas. Hofmeister [30] also states that Tanaka has found that when various tissues are acted on by calcium solutions, either *in vitro* or *in vivo,* only the fat tissue takes up calcium in appreciable amounts, and when solutions of calcium salts are injected into the peritoneal cavity there is formed a certain amount of calcium soaps in the subserous fat tissue. But that calcium soaps are formed in areas of necrotic fat is one thing, and that their formation is the usual first step in pathological calcification, is quite another.

This calcium soap hypothesis is certainly a most attractive one, and one that has long interested me greatly, especially as I supposed for some years that it was a discovery of my own, until I made a thorough search of the older literature. If true it settles all questions concerning pathological calcification, and leaves us with a closed chapter on this subject, certainly a condition most to be desired, and so for some nine years I have been endeavoring to prove that it is true, but without success.

The first point of attack naturally consists in seeking for calcium soaps in areas of calcification, especially during the early stages. There are technical difficulties involved which make the task almost impossible, except in cases in which the amount of calcium soaps is large, and on which I need not expatiate. It is sufficient here to state that I have never obtained convincing evidence of the presence of calcium soaps in areas of calcification of many kinds and at all stages, including some specimens, both experimental and natural, in which the process was known to be very early. A similar negative result was obtained by Baldauf,[31] who analyzed athero-

[30] Hofmeister: Ueber Ablagerung und Resorption von Kalksalzen in den Geweben, Ergebn. d. Physiol., 1910, ix, 429.

[31] Baldauf: The Chemistry of Atheroma and Calcification, Jour. Med. Research, 1906, xv, 355.

matous aortas, in which all stages of the process are occurring side by side in the numerous lesions. On the other hand, to prevent our case from being conclusively and finally settled, I have commonly found most minute traces of calcium in solutions obtained by prolonged extraction of calcified tissues with ether, ethyl alcohol and amyl alcohol at near the boiling point of each solvent. The amounts so obtained, however, were so extremely minute (usually 1 or 2 mg., sometimes less, from quantities of calcified tissues containing as much as 5 or 6 Gm. of calcium and 3 to 4 Gm. of fat) that it is very doubtful if this finding is of any significance. Experiments show that mixtures of calcium phosphate, calcium carbonate, fats and pure proteins, when submitted to a correspondingly vigorous extraction with the same solvents, may give off to the solvents corresponding minute quantities of calcium, since inorganic calcium salts are only relatively and not absolutely insoluble.

Therefore we are obliged to conclude that no appreciable amount of calcium exists in the form of soaps in any calcified areas that we have examined. To be sure, the objection at once presents itself that the amount of calcium soaps that is present at any one time during calcification may be too small to detect by chemical means, since according to the hypothesis the calcium is only transitorily in the form of soaps before going over to the inorganic form. This argument is, of course, unanswerable, if one wishes to insist on the sufficiency of infinitesimally minute quantities of calcium soap; and it is just this possibility alone that prevents us from saying positively that the calcium soap hypothesis of calcification is untenable. But we add to the improbability when we consider the very large size of the calcium soap molecule; in calcium stearate, for example, the calcium represents by weight but about one-fifteenth of the molecule; hence in an area of calcification containing 1 Gm. of calcium, the soap hypothesis demands that 15 Gm. of calcium soap shall have been formed during the process of calcification. On this basis the amount present at any one time in an area of rapid

calcification should be easily detected unless the rate of change of soap into phosphate and carbonate is much more rapid than we have any right to believe it can be. To take a specific example, I have analyzed a crumbling chalky calcified thrombus, which was formed as a result of an occlusion of the jugular vein by pressure from rapidly growing cancerous lymph-glands. From the history of the case it is improbable that this thrombus was over two months old, but to be liberal let us assume that it was 100 days old and that calcification had begun as soon as the thrombus was formed, instead of some days or weeks later, as is more probably the case. In this thrombus was found 0.730 Gm. of calcium. If this was laid down by first passing through a stage of soap formation there must have been, all told, about 11 Gm. of calcium soaps formed. Now if we assume that only the amount of calcium soap formed on a single day was present at any one time (which certainly represents a very rapid rate for this sort of chemical transformation to take place in so avascular an area as a large thrombus), there would be present 0.110 Gm. of calcium soaps, containing about 7 mg. of calcium. Nevertheless analysis of this thrombus yielded in the fat and soap fraction a mere trace of calcium, too small to be weighed, but probably less than 1 mg.; and even this trace presumably is to be ascribed to the solubility of inorganic calcium salts in the solvents used. Many other analyses of equally early stages of calcification give a similar lack of evidence of calcium soaps, and also show that the calcium is always present as phosphate and carbonate in the same proportions as in normal bone.

But even if calcium soaps are formed in a calcifying tissue, is it certain that the calcium so precipitated in the dead area would remain and be changed to phosphate and carbonate? In favor of this view we may refer to Jaeckle's calcifying lipoma, which contained, besides the 29.5 per cent. of calcium as soaps, much carbonate (28.61 per cent.) and phosphate (41.80 per cent.) of calcium, according to his analysis; this we may interpret as an illustration of incomplete transformation of calcium

soap into carbonate and phosphate.[32] I have performed a number of experiments (elsewhere described [24, 26]) to determine whether sodium soaps can become transformed within the body into calcium soaps and, if so, whether the calcium soaps are then replaced by calcium phosphate and carbonate. To simulate as closely as possible the conditions existing in calcifying tissues, in which the salts concerned are contained in an avascular mass of colloids, the soaps were imbedded in solidified agar jelly and implanted in the peritoneal cavity of rabbits. It was found that under these conditions a considerable proportion (30 to 40 per cent.) of the soap does become combined with calcium, and that a very small amount of inorganic salts of calcium (1 to 2 per cent.) appears. A strange feature of this process is that the same proportion of inorganic calcium is obtained whether the soaps have been implanted but a few days or several months, suggesting that possibly an equilibrium is here established. When calcium soaps are implanted the end results are much the same, the greater part of the soaps disappearing and a very small amount (1 to 2 per cent.) of carbonate and phosphate remaining, the two latter being always in the same ratio to each other as in normal bone.

From these results it may be considered as established that implanted sodium soaps do to some extent become changed into calcium soaps, but we are unable to consider the evidence clear as to whether the new-formed calcium soaps do or do not become changed into phosphate and carbonate. While these two inorganic salts are always found present in such implanted materials, yet the amount is so small that its presence might as well be explained as the result of simple infiltration of the

[32] Possibly one may consider as examples of a similar process the *Fettgewebesteine* described by O. M. Chiari (Ueber die herdweise Verkalkung und Verknocherung des subkutänen Fettgewebessteine, Ztschr. f. Heilk., 1908, xxviii [Suppl.], 1), which are found in the subcutaneous tissue of the lower extremities following arterial occlusion, but unfortunately chemical studies of this material have not been made.

implanted mass of soap and agar by calcium from the blood, irrespective of any reaction with the soaps. Speaking most strongly against the soap hypothesis, however, is the finding that most of the calcium which is introduced as soaps is absorbed, instead of being replaced by inorganic salts. This agrees entirely with my experiments on fat necrosis [33] in which it was found that in spite of early formation of calcium soaps in areas of experimental fat necrosis, complete healing and reabsorption may again take place in a few days or weeks. In other words, although fatty acids may cause calcium to be precipitated, yet they do not hold the calcium permanently in the tissues either as soaps or as inorganic compounds of calcium. This is presumably to be explained by the observation of Hofmeister [30] that the colloids of blood-serum enable it to keep large quantities of calcium soaps in solution. Also, as Hofmeister points out, pathological calcification is not always preceded by fatty degeneration, and, furthermore, if calcium soaps act catalytically, as has been maintained, calcification should be a most common, if not universal, occurrence, since it is as soaps that fatty acids are transported, and soaps are often, if not always, present in the tissues without calcification taking place.

The affirmative side of the question has been most strongly supported by Klotz,[35] largely on a basis of microscopical evidence. This consisted chiefly in finding in calcifying areas granules staining "pinkish yellow" with Sudan III, which Klotz states distinguishes them as soaps because neutral fat stains golden red. By staining with Von Kossa's silver nitrate stain for calcium salts, granules staining black were found in the same location as the granules which stain "yellowish pink" with Sudan III, and hence Klotz considered that this "suggests strongly that we are dealing with a deposit of calcium soap." Unfortunately for this doctrine, calcium soaps have been shown

[33] Wells: Experimental Fat Necrosis, Jour. Med. Research, 1903, ix, 70.

[35] Klotz: Studies on Calcareous Degeneration, Jour. Exper. Med., 1905, vii, 633.

by Baldauf [36] not to stain at all with Sudan III,[37] and in the second place in Von Kossa's silver nitrate method the silver reacts with phosphoric acid and not with calcium; hence the results of these observations merely show that insoluble phosphates are present in the earliest stages of calcification, and do not give any evidence whatever that calcium soaps or any other kinds of soaps are present and demonstrable by microchemical measures. We can, indeed, scarcely hope to obtain microchemical evidence of calcification if chemical procedures are unavailing; my analyses show that if calcium soaps are present at all in calcifying tissues, they can represent not over one-thousandth of the total calcium, and to distinguish by the microscope such minute quantities of a calcium soap from all the remaining 999 parts of inorganic calcium with which it is mingled, is evidently impossible. Other features of this work, which time does not permit me to take up at length, are also open to criticism, and in the final analysis I can find in it no satisfactory support for the calcium soap hypothesis. Hofmeister,[30] in a recent review of the literature of calcification, also refuses to accept this work as establishing the calcium soap theory of calcification.

A point that should be especially mentioned is that there is no reason whatever to believe that the formation of normal bone from cartilage takes place through a stage of soap formation, hence the acceptance of the soap theory would compel us to separate sharply ossification and calcification, whereas most of the evidence indicates the unity of these two processes.

[36] Baldauf, Leon K.: An Investigation of the Nature of Proteid-Soap Compounds and of the Staining of Pure Fats and Lipoids by Schlarlach R and Sudan III, Jour. Am. Med. Assn., 1907, xlix, 642.

[37] To quote from Baldauf, "Neither calcium nor sodium soaps, palmitic nor stearic acids, will at room temperature take the stain with Sudan III or Scharlach R, hence the material staining by these substances, which Klotz described as occurring at certain stages of calcareous degeneration, cannot be any of these compounds. In all probability it is a mixture of oleic acid, triolein and lecithin." Aschoff (Zur Morphologie der lipoiden Substanzen, Beitr. z. path. Anat. u. z. allg. Path. (Ziegler's), 1909, xlvii, 1) also takes exception to the supposed differentiation of soaps by Sudan III.

To recapitulate, then, we can say that while there is some reason from a theoretical stand-point to support the idea that fatty acids formed in necrotic areas may combine calcium from the blood and precipitate it in the tissues where it may later undergo transformation into carbonate and phosphate, yet that this is the ordinary process by which calcification occurs, is unproved. Other considerations, to be expressed later, make it seem improbable.

Besides fatty acids, few other organic *Kalkfänger* have been suggested. Special proteins with a specific affinity for calcium, or products of protein hydrolysis, such as albumoses, have been thought of, but no proof of their existence has been brought forward, nor has their existence been made probable.

Another possible method of precipitation of calcium salts would be by an *increased alkalinization* of the degenerating tissues, which might cause mono- and dicalcium phosphates and bicarbonate of calcium to be converted into the less soluble basic salts and precipitate from the blood. That a change in reaction can be a sufficient cause for precipitation of calcium salts from the blood into normal tissues, is made probable by that unusual type of calcification which was first described by Virchow under the title of "metastatic calcification."

METASTATIC CALCIFICATION

Here we find calcium salts deposited throughout the body in what seem to be perfectly normal tissues, but especially in the lungs, the kidneys and the gastric mucosa. Of the twenty-nine cases recorded in the literature [38] in all but four there was

[38] Askanazy (Beiträge zur Knochenpathologie, Festchr. f. affe, Brunswick, 1901; quoted by Hofmeister; see Note 30) in 1901 collected reports of twenty-one cases, to which Hofmeister (see Note 30) added two more. In addition to these I have noted cases reported by Hedinger (Ueber Verkalkung der Leber, Corrsbl. f. Schweiz. Aerzte, 1909, xxxix, 833), Pari (Ueber einen Fall von Kalkinkrustation der Lungen mit Fragmentation der elastischen Fasern, Virchow's Arch. f. path. Anat., 1910, cc, 199), Versé (Ueber ausgedehnte Verkalkungen der Lungen, Verhandl. d. Deutsch. path. Gesellsch., 1910, xiv, 281), Jadassohn (Ueber Kalkmetastasen in der Haut, Arch. f. Dermat.,

demonstrated some extensive destructive disease of the bones,[39] supporting the assumption made by Virchow that the primary cause of the disease is an overloading of the blood with calcium salts. Recent experimental work by Hofmeister and Tanaka leaves no question that this assumption is correct, for they found it possible to cause extensive and typical metastatic calcification by intraperitoneal injection of soluble calcium salts into rabbits.[40] The fact that the deposition of calcium takes place most often in these three tissues (lungs, stomach and kidneys), where otherwise calcification is not commonly seen independent of local lesions, such as tubercles, thrombi, etc., is of special significance, for as has been pointed out elsewhere (Askanazy,[38] Wells [25]) in these three tissues we have the three chief places in

1910, c. 317), Lazarus and Davidsohn (Hirnhautsarkom mit zahlreichen Kalkmetastasen, Ztschr. f. klin. Med., 1906, lx, 314), and Tschistowitsch and Kolessnikoff (Multiples diffuses Myeloma mit reichlichen Metastasen in die Lungen und andere Organe, Virchow's Arch. f. path. Anat., 1904, cxcvii, 112). This does not include the remarkable cases of generalized subcutaneous calcification of unknown etiology, recently discussed by l'Hermitte (La calcinose généralisée et ses formes anatomiques interstitielle et sous-cutanée, Sem. méd., 1910, xxx, 553).

[39] In many cases nephritis also exists, which Hedinger (see Note 38) believes may be a factor by interfering with calcium excretion. However, since normally most of the calcium excretion takes place through the bowel, it seems improbable that disease of the kidney can have much influence on the calcium content of the blood, although Erben (Ueber die chemische Zusammensetzung des Blutes, Ztschr. f. klin. Med., 1903, l, 441) did find an increased amount of calcium in the blood in nephritis.

[40] A human case quite comparable to these experimental calcifications has been reported by Thayer and Hazen (Calcification of the Breast Following a Typhoid Abscess, Jour. Exper. Med., 1907, ix, 1). A typhoid patient was given during eleven days, on account of hemorrhages, 132 Gm. calcium lactate by mouth, and 5 Gm. calcium chloride as a subcutaneous injection. At the site of the latter there developed an abscess, in the margins of which some days afterward a deposition of calcium salts took place, later becoming absorbed under a diet free from carbohydrates. The form in which the calcium was held was not determined, beyond noting that it dissolved in HCl without effervescence.

the body where acids are excreted. In the lungs we have bicarbonates giving up CO_2 and passing on in the blood as carbonates; in the stomach HCl is excreted, and in the kidneys acid phosphates are excreted by a reaction which leaves basic phosphates and carbonates in the blood and tissues. We therefore believe that the coincidence of the location of the calcium deposits and the acid-excretion function of the tissues leaves little or no room for doubt that the precipitation of calcium occurs in this condition of metastatic calcification because calcium salts are slightly less soluble in the more alkaline fluids present in these tissues.[41] The histological findings in the calcified areas of the stomach are remarkably conclusive as to this point for, as Hofmeister points out, the calcium deposits are limited to the interglandular tissue about the upper portion of the glands of the fundus—in other words, exactly corresponding to the location of the parietal cells which are (as the observations of Mabel Fitzgerald[42] show finally) the cells which secrete the hydrochloric acid. In some cases of bone absorption we also find wide-spread calcium deposits in the walls of the heart, arteries and capillaries, independent of degenerative changes in the tissues of these vessels; thus Lazarus and Davidsohn[38] described extensive internal calcification of the left auricle, which is expressly noted to have come from the blood within the auricle, and not from the vessels of the heart wall;

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[41] There seem to have been no quantitative analyses made of the deposits in metastatic calcification. Pari (see Note 38) could find no P_2O_5 by microchemical methods. Jadassohn (see Note 38) found both carbonate and phosphate in the skin metastases of his case. Hofmeister in experimental metastatic calcification found calcium phosphate and a little carbonate, no matter what salt had been injected. Hedinger (see Note 38) in calcified areas in the liver found P_2O_5, but observed no effervescence on addition of acid. Versé (see Note 38) found in his case both carbonate and phosphate. Tschistowitsch and Kolessnikoff (see Note 38) found the ash of the lungs, kidneys and aorta in their case to contain greatly increased amounts of Ca and P_2O_5, but no analysis of isolated calcific material was made.

[42] Fitzgerald, Mabel: The Origin of Hydrochloric Acid in Gastric Tubules, Proc. Roy. Soc. London, 1910, lxxxii, 346.

Versé[38] found, in a case of leukæmia, deposits in the lungs, pulmonary veins, and most extensively beneath the endocardium of the left side of the heart; Jadassohn[38] observed deposits in the left auricle in a case of myocarditis, and Küttner[43] in a case of rarefying osteitis noted generalized arterial deposits in and on the intima, without deposits present in the media or in the vein walls. In these cases the explanation is somewhat similar to that given above for the visceral deposits, for here, it will be noted, the calcification has taken place in the walls of those vessels whose contents have the smallest amount of CO_2. In other words, the carrying of calcium salts by the blood is, at least in the cases of excessive bone absorption, largely dependent on the carbon dioxide of the blood. Whether this is the normal condition or not will be considered later. In this place we are concerned with the question of whether a similar process of alkalinization takes place in calcifying and ossifying tissues, to cause precipitation in them of calcium salts brought in the tissue fluids. One can readily imagine that ammonia set free during autolysis in necrotic areas might cause such an alkaline reaction, but the evidence which we have speaks rather for the development of an excess of acids in such areas. Not only are acids supposed to be formed during autolysis of tissues *in vitro,*[44] but certain histological evidence is in favor of the view that calcification takes place in acid tissues. In the first place cartilage, which is the favorite site of calcium deposition, takes on basic stains, implying that it is of an acid reaction which probably is caused by the chondroitin-sulphuric acid. It is also said that in rickets osteoid tissue which is not capable of calcification fails to assume this basophilic character. In areas of calcification after removal of the calcium salts we also

[43] Küttner: Ein Fall von Kalkmetastase, Virchow's Arch. f. path. Anat., 1872, lv, 521.

[44] The investigations of Jackson, Wolbach, and Saiki (The Rate of Autolysis and the Appearance of Gases and Acids in the Autolysis of So-Called Sterile Livers of the Dog, Jour. Med. Research, 1909, xxi, 281) have opened the question as to how much of the acidity observed in so-called aseptic autolysis depends on hitherto unrecognized anäerobic bacteria, and how much upon the autolysis itself.

find that the ground substance shows the same basophilic (therefore presumably acid) character. Finally, Schmidt[45] has noted that iron pigment present in splenic infarcts is rapidly removed; he does not explain this, but as iron salts are uniformly insoluble in alkalies and soluble in very weak acids, we may consider this fact to be a natural experiment indicating the development of acids in necrotic infarcted areas, which are so prone to calcify. The well-known tendency of muscle fibres to calcify in the vicinity of recent wounds may perhaps also be placed in this category, since there is much evidence of an accumulation of lactic acid in injured muscle fibres[46] and this might be neutralized in part by calcium from the blood; in any case we here again find calcification taking place in acid rather than in alkaline tissues.

We must conclude, therefore, that while in conditions of oversaturation of the blood with calcium salts, either from bone absorption or experimental injection, the presence of a local alkalinity or, perhaps more accurately stated, a decreased amount of CO_2 in the fluids and tissues, may determine the deposition of calcium in otherwise normal structures, yet it is not probable that any such reaction is responsible for the ordinary deposition of calcium in ossifying cartilage or in necrotic tissues. We must admit, however, that in this case also the negative evidence is not absolutely conclusive, and we cannot leave out of our considerations the possibility that local changes in tissue reaction may determine calcium deposit. Especially is this reservation necessary since we have abundant evidence that the occurrence of both normal and pathological calcification depends in large measure on the amount of calcium present in the blood. Animals starved of calcium develop, if adult, an osteoporosis, and if young a pseudo-rickets. Experimental calcification in the kidneys is favored by a calcium-rich diet or

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[45] Schmidt, M. B.: Ueber Schwund des Eisens in der Milz, Verhandl. d. deutsch. path. Gesellsch., 1908, xii, 271.

[46] Wells: The Pathogenesis of Waxy Degeneration of Striated Muscles, Jour. Exper. Med., 1909, xi, 1.

injections of calcium salts (Von Kossa [47]). Rabbits are the most favorable animals for use in studies of experimental calcification because of the high calcium content of their blood, but if they are fed on a calcium-poor diet, calcification of the arteries will not follow adrenalin injections (Loeper and Boveri [48]), nor can experimental atheroma be produced in pregnant rabbits whose calcium supply is being utilized by the fœtus. Therefore it is evident that both normal and pathological calcification are dependent at least to some extent on the amount of calcium in the blood, which brings us to the subject of the conditions of the solution of calcium by the blood.

THE SOLUTION OF CALCIUM BY THE BLOOD

In the blood we have present the anomalous condition of calcium in solution in a fluid containing carbonates, phosphates, and sulphates, any one of which would throw it down in the test-tube. According to Abderhalden the red corpuscles contain no appreciable amount of calcium, which is all in the serum, the amount of CaO varying but little in mammals,[49] being from 0.0110 to 0.0131 per cent. Birds' blood contains similarly 0.007 to 0.02 per cent. of CaO (Hiller[4]). Water will hold in solution but about 0.0079 per cent. of tri-calcium phosphate, the form of calcium phosphate which is assumed to be present in the blood because of the alkali carbonate it contains; therefore tri-calcium phosphate is normally contained in the blood of various mammals *in two to four times as large an amount as it can be held in solution in water.* To account for this phenomenon we have at least two possible agencies, the colloids and the CO_2. It is a well-known property of colloids to keep otherwise insolu-

[47] Von Kossa: Ueber die im Organismus künstlich erzeugbaren Verkalkungen, Beitr. z. path. Anat. u. z. allg. Path. (Ziegler's), 1901, xxix, 163.

[48] Loeper and Boveri: La chaux et les artères, Presse Méd., 1907, xv, 401.

[49] Kossa gives for rabbits' serum the incredibly high figure of 0.150 per cent. while Abderhalden found in the same animal but 0.0116 per cent.

ble substances in solution or in suspension, and the property varies definitely with different colloids, which fact is taken advantage of in the study of the characteristics of colloids by determination of their *Goldzahl*. This *Goldzahl* refers to the number of milligrammes of an emulsion colloid (such as the proteins) which is required to prevent precipitation of a characteristic suspension colloid (colloidal gold) by a standard quantity of sodium chloride.

Colloids maintain the solution of crystalloids in the following manner: in a solution containing crystalloids, which are in solution, and colloids, which are in suspension, we have really a solution with two phases, colloid and water. In such two-phase systems part of the crystalloid is concentrated at the surfaces of the colloid particles where the two phases meet, and held here in higher concentration than elsewhere. Consequently the water phase is relieved of part of its load of crystalloid, and is able to take up still more before becoming saturated, so that the total amount of crystalloid dissolved by an aqueous colloidal solution is greater than the amount that can be dissolved by the same quantity of water.

Another possibility is the presence of soluble ion-protein compounds in the blood, but the fact that all the calcium can be readily precipitated from the blood by ammonium oxalate is, according to Hofmeister, conclusive evidence that the calcium is not carried dissolved as such ion-protein compounds. Furthermore, Michaelis and Rona have shown that in milk the calcium is in true solution and not in colloidal solution or suspension, and the same is probably equally true of the blood.

Since the solution and precipitation of calcium by blood-serum is a typical case of colloidal solution of crystalloids, we must carry the above facts into all our considerations of calcification, as Schade [50] has done with such success in the related field of concrement formation.

[50] Schade: Beiträge zur Konkrementbildung, München. med. Wchnschr., 1909, lvi, 3. Zur Enstehung der Harnsteine, Kolloide Ztschr., 1909, iv, 175 and 261.

Pauli and Samac[51] have studied the solubility of calcium salts in dialyzed serum, and contrast the results with their solubility in water and in gelatin, as shown in Table I.

TABLE I.—SOLUBILITY OF CALCIUM SALTS

	Water	Percent. soluble in Serum	1.5 Percent. Gelatin
$CaSO_4$	0.223	0.226	0.295
$Ca_3(PO_4)_2$	0.011	0.021	0.018
$CaCO_3$	0.004	0.023	0.015
SiO_2	0.023	0.030	0.027
Uric acid	0.040	0.057	....

Hofmeister[30] found that when calcium chloride and disodium phosphate react in an alkaline horse-serum there is no precipitation until the concentration of newly-formed tri-calcium phosphate is 0.15 per cent., which is five to ten times greater than the amount of this salt which is present in normal horse-serum.

We may therefore assume that the colloids of the blood are adequate to account, at least in part, for the power of the blood to carry calcium salts in solution. If all the calcium of the blood, however, exists as phosphate and carbonate, the degree of solubility which Hofmeister and Pauli found for these salts in serum is not nearly so great as would be required to account for the amount of calcium (1 to 2 per cent. of dry weight or about 0.15 per cent. of entire blood) which Von Kossa found in the blood of rabbits. The 0.014 per cent. of CaO found in dog's blood by Boggs[52] would, if in the form of tri-calcium phosphate, amount to about 0.026 per cent., which is a little more than the amount of this salt (0.021 per cent.) which Pauli and Samac found soluble in dialyzed serum free from CO_2 and salts. We need, therefore, to consider the influence of CO_2, which is

[51] Pauli and Samac: Löslichkeitsbeeinflussung von Elektrolyten durch Eiweisweiskörper, Biochem. Ztschr., 1909, xvii, 235.

[52] Boggs: Variations in the Calcium Content of the Blood Following Therapeutic Measures, Johns Hopkins Hosp. Bull., 1908, xix, 201. Bunge: Kochsalzgehalt des Knorpels und das biogenetische Grundsatz, Ztschr. f. physiol. Chem., 1899, xxviii, 452.

known to be capable of dissolving calcium, even as it exists in bone, converting it into the relatively soluble acid calcium carbonate and at the same time causing the formation of the mono- and di-calcium phosphates, both of which are more soluble than the tri-calcium phosphate. It is, indeed, probable that it is the CO_2 which accomplishes the resorption of dead bone in the living body, and perhaps also the normal resorption of bone in the various conditions in which this process takes place. It was long ago demonstrated by Maly and Donath that CO_2 in solution will dissolve calcium from pieces of bone and that $NaHCO_3$ will not dissolve it, and Hofmeister and Tanaka [30] have studied quantitatively this solubility *in vitro* and *in vivo,* finding that pieces of ivory are absorbed most rapidly in tissues whose metabolism is the most active and where, by inference, there is the most CO_2 production.[53] The influence of CO_2 on the solution of calcium salts has been studied most extensively by Barillé,[54] who concludes that calcium is carried in the blood in the form of a definite compound, a carbon-phosphate of calcium. This author finds that 1 litre of water saturated with CO_2 at 10 kg. pressure can dissolve 0.923 Gm. of tri-calcium phosphate, which forms a solution of an unstable compound, supposedly according to the following reaction:

$$Ca_3P_2O_8+4H_2CO_3=H_2O+P_2O_8Ca_2:2CO(CO_3H)Ca$$

(tri-basic calcium carbon-phosphate)

If this solution is evaporated to dryness the salt decomposes, yielding 0.975 Gm. of precipitate, which consists of 0.709 Gm. $CaHPO_4$ and of 0.260 Gm. $CaCO_3$. Only those bases which form bicarbonates (namely K, Ka, NH_4, Ca, Mg and Ba) can form carbon phosphates, all of which are unstable and decompose to form a biphosphate and a bicarbonate. Phosphates of other

[53] According to Morpurgo and Satta (Sur quelques particularités de l'autolyse, Arch. ital. de biol., 1908, xlix, 380) calcium may be dissolved from bone tissue during autolysis of bone, but by what process they do not determine.

[54] Barillé: Carbonphosphates tricalcique, Jour. de pharm. et chim., Jan. 1 to March 16, 1904, series 6; 1910, series 7, i, 342, 377.

bases form simple solutions in CO_2 and water. Barillé believes that these six bases, or rather the first five, which are normal constituents of the body, all exist in the blood in the form of the carbonphosphates, and when the CO_2 is given off, or neutralized by other bases, the mixture of carbonate and phosphate is precipitated. If the precipitation takes place in an alkaline medium or in one with acid-neutralizing properties, such as the blood, the phosphate comes down as the tri-calcium phosphate, as in bones, intestinal concretions, atheromatous vessels, etc.; if in an acid medium, as in normal urine, we have the dicalcic phosphate. Analyses of different sorts of calcific deposits give results agreeing with this. Now if we recur to Hofmeister's studies of the precipitation of calcium when dissolved to excess in the body fluids, or to the phenomena of metastatic calcification, especially such cases as those of Versé, Jadassohn and Küttner, where we have precipitation of calcium in and on the intima of the vessels containing the least CO_2, we find much support for this hypothesis of Barillé's. Furthermore, there is the well-known fact that, no matter how sclerotic the walls of the veins may become, they rarely, if ever, calcify so long as there is venous blood rich in CO_2 flowing through them. As soon as they are occluded, however, calcification occurs readily enough (*e.g.*, phleboliths). We may consider that the solution of calcium in the blood is as the carbon-phosphate, in part or in whole, and that under ordinary conditions the precipitation of calcium is prevented by the colloids, no matter how completely the CO_2 is removed from the blood, for, as Hofmeister's experiments as well as general experience show, a watery solution of colloids can maintain a much higher percentage of a crystalloid in solution in the presence of precipitants than it can dissolve from masses of the precipitated salt; *e.g.*, Hofmeister found that when calcium phosphate was precipitated in serum there was no precipitate until the concentration reached 0.15 per cent., while Pauli and Samac had found that serum dissolves but 0.023 per cent., one-seventh as much, of solid tri-calcium phosphate. Only in such extreme conditions of over-saturation of the blood with calcium as occur after experimen-

tal injection of calcium salts, or rarely when great quantities of bone are being rapidly absorbed by tumors and other active disease processes, does it become impossible for the blood to hold in solution the carbon-phosphate, which is then precipitated, especially in those places where the CO_2 is given off (the lungs) or neutralized by acid excretion (stomach and kidneys) or where the CO_2-rich and colloid-poor lymph gives up its CO_2 to the arterial blood (the intima of the arteries). This hypothesis receives further support when we consider that tricalcium carbon-phosphate was found by Barillé to be decomposed on evaporation of its aqueous solution into seventy-seven parts of di-calcium phosphate and twenty-three parts of calcium carbonate. Now, in potentially alkaline solutions, such as the tissue fluids, the di-calcium phosphate would go over to the tri-calcium phosphate, and from seventy-seven parts of di-calcium phosphate would be formed 87.7 parts of the tri-calcium salt, or exactly the proportion of calcium phosphate which all my analyses have shown to prevail in bone and calcified tissues. It may also be mentioned that Hofmeister and Tanaka found that in experimental metastatic calcification, no matter what soluble salt of calcium they had injected, the deposits always consisted "aus Calciumphosphat mit wenig Calciumcarbonat."

THE RELATION OF THE COMPOSITION OF CALCIUM SALTS IN BLOOD AND TISSUE

It is indeed unfortunate that erroneous methods of analysis have kept us so long from appreciating the constancy and identity of composition of both normal and pathological deposits of calcium within the tissues, for this is an important consideration in our problems. If we take into account, however, the conditions of circulation in the body, we at once find that it would not be possible for anything except a practically constant and identical composition of bone and calcified tissue deposits to exist for any length of time. The same blood which furnishes the calcium salts to the calcifying tissues is also passing and repassing through great areas of capillaries within the

bone-tissue, where are present large quantities of calcium phosphate and carbonate that are in a proportion which is practically constant, not only for the bones of the same animal, but also for the bones of all mammals as far as they have yet been investigated. In both the normal and pathological tissue calcium is being given off to or taken up from the surrounding fluids according to the laws of solution tension and of chemical and osmotic equilibrium, and therefore no matter what the composition of the pathological deposit may have been originally, there can be no question that eventually it will become the same as that of the bone; and even more certainly if the calcium salts are deposited from the blood the resulting precipitate cannot from the beginning be very different from what it is in the calcium storehouse, the bones. Direct experiments

TABLE II.—RESULTS OF IMPLANTATION OF CALCIUM SALTS

Salt implanted	Days before removal	$Ca_3(PO_4)_2$ in inorganic Ca salts per cent.	$CaCO_3$ in inorganic Ca salts per cent.
Calcium phosphate	98	76.3	8.8
	160	92.6	4.6
	197	61.2	38.8
Calcium carbonate	90	1.15	98.85
	190	8.1	91.9
	200	9.8	90.2
Calcium sulphate	80	94.1	5.9
	190	91.8	8.2
	220	93	7

made by J. H. Mitchell and myself [26] have shown that mixtures of calcium phosphate and sodium carbonate, and of sodium phosphate and calcium carbonate, when shaken together for some time come to an equilibrium which is quite the same in either case, the calcium carbonate and phosphate being present in the precipitate and in the solution according to their relative solubility.

Likewise when we implanted masses of calcium carbonate or of calcium phosphate into animals we found that in time phosphate is taken up by the carbonate, and carbonate by the phos-

phate (see Table II), so that even in so unnatural and extreme a condition as this artificial implantation, in due time we should undoubtedly have present the salts in the same proportion as in the normal bones. Hofmeister and Tanaka[30] have independently obtained corroborative results.

In pathological calcification the deposit is from the first (except in the case of calcifying fat tissue) undoubtedly a mixture of carbonate and phosphate in nearly or quite the standard proportions. That the composition of the bone itself is constant we must ascribe, not to any special combination of ions of calcium, magnesium, P_2O_5 and CO_2 with the stroma, but to the relative solubility of these salts in the blood-stream. In support of this idea may be cited the experiments of Hofmeister and Tanaka, who found that if $Ca_3(PO_4)_2$ was acted on for twelve days at 37° C. by a solution composed, in imitation of the blood, of 0.9 per cent. NaCl, 0.1 per cent. Na_2CO_3, and 0.1 per cent. $NaHCO_3$, the precipitate contained 85.2 per cent. calcium phosphate and 12.75 per cent. calcium carbonate, *i.e.*, the same composition as bone. If a different concentration of alkali was used the ratio of phosphate to carbonate could be changed; the more alkaline the solution the more calcium carbonate in the precipitate. Just as the blood is of nearly constant, yet incessantly varying composition, so too the bone salts may vary within very narrow limits, as they are laid down or taken up according to the relative amount present in the blood, not only of calcium salts, but also of CO_2, of colloids, and of other salts, each of which modifies to some extent the solubility of the calcium salts. For example, we may greatly modify the composition of the bone by feeding food poor in calcium and rich in magnesium and strontium,[55, 56] substituting the latter two

[55] Stoeltzner, Helene: Ueber den Einfluss von Strontiumverfütterung auf die chemische Zusammensetzung des wachsenden Knochens, Biochem. Ztschr., 1908, xii, 119.

[56] Lehnerdt: Zur Frage der Substitution des Kalziums im Knochensystem durch Strontium, Beitr. z. path. Anat. u. z. allg. Path. (Ziegler's), 1909, xlvi, 468; 1909, xlvii, 215.

bases for a considerable proportion of the calcium [57] and also, as is well known, calcium may be withdrawn to a large extent to neutralize acids in acidosis, experimental or pathological, or to meet drains on the bases from pancreatic and biliary fistulæ (Babkin,[58] Looser,[59] Seidel [60]). Conversely, excess introduction of calcium can lead to a certain amount of deposition of calcium in the bones (Goitein [61]), although most of the excess is excreted in the urine and fæces.

It has been found that there is a regular increase in the ratio of phosphate to carbonate in the bones with advancing years. Wildt [62] gives the figures of Table III for the ash of rabbits' bone at different ages.

TABLE III.—ASH OF RABBITS' BONES AT DIFFERENT AGES

	At birth	1 mo.	6 mo.	1 yr.	4 yrs.
Ca phosphate	86.04	85.87	84.47	82.45	82.25
Ca carbonate	8.30	9.09	11.23	12.98	12.86
Mg phosphate	3.01	2.66	2.29	1.99	1.81

Similar results have been obtained by Graffenberger [63] and by Gabriel,[64] the latter of whom also notes slight constant dif-

[57] However, hens are unable to substitute magnesium or strontium for calcium in their shells (see Note 1).

[58] Babkin: Material zur experimentellen Pathologie und Therapie der Hunde, Zentralbl. f. Stoffwechsel, 1910, xi, 561.

[59] Looser: Ueber Kochenveranderungen bei chronischen Fisteln der grossen Verdauungsdrüsen, Verhandl. d. deutsch. path. Gesellsch., 1907, xi, 291.

[60] Seidel: Permanente Gallenfistel und Osteoporose beim Menschen, München. med. Wchnshr., 1910, lvii, 2034.

[61] Goitein: Ueber den Einfluss verschiedener Ca- und Mg-Zufuhr auf den Umsatz und die Menge dieser Stoffe im tierischen Organismus, Arch. f. d. ges. Physiol. (Pflüger's), 1906, cxv, 118.

[62] Wildt: Zusammensetzung des Knochen des Kaninchens in verschiedenen Altersstufen, Landwirtsch. Versuchsstat., 1872, xv, 404.

[63] Graffenberger: Ueber die Zusammensetzung der Kaninchenknochen im hohen Alter, Landwirtsch. Versuchsstat., 1891, xxxix, 115.

[64] Gabriel: Chemische Untersuchungen über die Mineralstoffe der Knochen und Zähne, Ztschr. f. physiol. Chem., 1893, xviii, 257.

ferences in the ash (by the glycerin method) of bones of different vertebrates, as follows:

TABLE IV.—PHOSPHORUS PENTOXID AND CALCIUM DIOXIDE IN BONES OF MAN, THE OX AND THE GOOSE

	Human	Ox.	Goose
P_2O_5	36.65	37.46	38.19
CO_2	5.86	5.06	4.11
Total	42.51	42.52	42.30

These regular variations in the proportion of calcium carbonate and phosphate present in normal bones of different ages and species, dispose entirely of the hypothesis of Hoppe-Seyler that there is in bones a complex chemical compound of calcium phosphate-carbonate of definite composition.

It is probable that we shall find these variations in the salts of bone to be dependent on differences in the composition of the blood which affect its solvent power for calcium salts. In osteomalacia, the calcium is taken out of the bones in the same ratio of phosphate and carbonate as it there exists (Levy [65]), fully supporting the hypothesis that the salts exist in the bones according to the laws of solubility.

THE PHYSICAL CHEMISTRY OF CALCIFICATION

Granting that the work of Hofmeister, Pauli, Barillé and others has given us some rational understanding of how the calcium is carried in the blood and why the composition of the deposits of calcium is constantly what it is, yet we have been unable so far to explain why the deposition of calcium salts occurs in the places where it does occur. I have failed, as Pfaundler had before, to find any chemical substance in these places which will account for the deposition of calcium, and the evidence at hand indicates that the calcium is carried in and precipitated from the blood as a mixture of phosphate and

[65] Levy: Chemische Untersuchungen über osteomalacische Knochen, Ztschr. f. physiol. Chem., 1894, xix, 239.

carbonate, hence rendering unwarranted any hypothesis of a primary chemical combination of the calcium of the blood with any tissue element. Only in metastatic calcification have we secured evidence of chemical processes which can account for the calcium precipitation. Therefore, finding no clue on the chemical side we naturally turn to the physical aspects, and here we obtain some interesting evidence. The first systematically to consider this possibility seems to have been Pfaundler,[66] who based his work on the earlier observations of Hofmeister and Spiro, that disks of gelatin placed in solution containing various crystalloids are able to absorb some and not others; that is, colloids exert a specific absorption affinity for crystalloids. Pfaundler observed that cartilage immersed in solutions of calcium chloride takes up more calcium than chlorine, suggesting to him the existence of a specific ion affinity. Other tissues were also found to exhibit a similar but less marked affinity for calcium. However, many of the results obtained by Pfaundler were based on a miscalculation,[67] and I have been unable to secure any positive evidence that there is any marked affinity of cartilage *in vitro* for calcium ions, although it is probable that cartilage does absorb calcium salts to some extent, just as it and many other colloids absorb many different crystalloids; *e.g.*, cartilage absorbs uric acid (Almagia,[68] Brugsch and Citron [69]). Pfaundler was unable to ascribe calcification to this absorption affinity, however, because crystalloids absorbed

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[66] Pfaundler: Ueber die Elemente der Gewebsverkalkung und ihre Beziehung zur Rachitisfrage, Jahrb. f. Kinderh., 1904, lx, 123.

[67] The results of Pfaundler's experiments with pseudo-rachitic limbs are even more doubtful, because the injection of calcium chloride was preceded by injections of NaCl solution to wash out the blood, and apparently the author has not taken into account the NaCl which would be absorbed by the tissues from the wash water and then given back to the $CaCl_2$ solution.

[68] Almagia: Ueber das Absorptionsvermögen der Knorpelsubstanz für Harnsäure, Beitr. z. chem. Physiol. u. Path. (Hofmeister's), 1905, vii, 466.

[69] Brugsch and Citron: Ueber die Absorption der Harnsäure durch Knorpel, Ztschr. f. exper. Path. u. Pharm., 1908, v, 401.

in vitro are washed out readily and not held firmly in the colloids; furthermore, adsorption of calcium seems to be a general colloidal property, not at all limited to the cartilage, but exhibited by muscle and other tissues. Also, as Aron[10] has pointed out, these experiments concern adsorption of calcium ions, while in calcification we have to do with the accumulation of neutral salts. Hofmeister, however, comes to the rescue with an interesting suggestion, which may be stated as follows.

We may imagine the cartilage causing by its absorptive powers an accumulation within itself of calcium salts from the blood, up to or near the limit of saturation. Now the amount of these salts which the tissue fluids can hold in solution depends, as we have seen, partly on the amount of CO_2 which is

TABLE V.—MILLIGRAMS OF CALCIUM OXIDE IN EACH GRAM OF DRY TISSUE

	Control	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks	14 wks.
Muscle	±0.4	....	4.7	6.4	11.3	....	....
Fat	trace	....	1.0	...	3.3	4.7	....
Spleen	∓0.3	....	3.3	...	8.4	....	12.0
Thymus	trace	....	...	8.7	....	....	12.0
Cartilage ...	3.3	15.5	...	...	124.6	162.0	154.0

present, and this is a variable quantity. If the amount of CO_2 is reduced there will result a precipitation of part of the calcium salts, thus restoring to the cartilage the power of adsorbing more calcium salts whenever the tissue fluids come to it with a higher degree of saturation with calcium salts and CO_2; this process can be imagined as going on until the cartilage is entirely incrusted with calcium deposits so that its adsorptive affinity is entirely lost. This hypothesis possesses one great advantage, in that it leaves out of consideration any activity on the part of the cartilage itself, for my own experiments have conclusively shown that calcium deposition takes place in cartilage more than in other tissues *independent of any "vital action"* on the part of the cartilage.[25] As Table V shows, when various tissues, sterilized and killed by heating to 100° for half an hour on each of two days, are left in the

abdominal cavity of rabbits for some time, only the cartilage takes up any considerable amount of calcium, but the cartilage comes to contain so much calcium that this is visible to the naked eye as thin scales as large as 1 to 2 mm. in width.

Here there can be no question of any cellular activity on the part of the killed cartilage, and as the calcium was found to be combined with phosphoric acid in quite the same proportion as in bone, we may exclude such calcium-ion adsorption as Pfaundler considers. I know of no reason, however, why Hofmeister's theorization cannot be applied here. The objection may be raised that in the normal body some sorts of cartilage ossify and some do not, which may be considered as evidence of a difference in physiological activity, since no structural difference can be found which accounts for the difference in behavior. I have found,[33] however, that if cartilages which normally do not ossify (tracheal and costal cartilages) be implanted in a similar way, they do not take up nearly so much calcium as the epiphyseal cartilages, as seen in Table VI.

TABLE VI.—MILLIGRAMS OF CALCIUM OXIDE PRESENT IN THE ENTIRE IMPLANTED MATERIAL

Weeks	2.	4.	6.	8.	10.	12.	14.	16.	18.
Costal	..	0.5	..	1	6	15	...	abs.	...
	..	..	..	5	12	abs.	...	...	...
	..	..	..	4	..	...	...	...	...
Tracheal	5	..	10	1.8	..	20	28	46	...
	..	..	19	..	..	...	...	...	...
	..	..	3	..	..	...	...	...	...
Epiphyseal	..	10	37	54	134	109	167	...	151
	..	..	37	88	117	128	...	...	...

Therefore we must admit that the characteristic difference which these cartilages exhibit in respect to their tendency to lay on calcium salts is not in any way dependent on any physiological or vital activity of their cells, for it is exhibited just as strikingly by the dead cartilage. We have been unable to detect any chemical differences in the three sorts of cartilage sufficient to account for this difference in behavior,[33] and we have not found any corresponding variation in their adsorptive affinity for cal-

cium salts *in vitro.*[22] It must be borne in mind, however, that all forms of hyaline cartilage are prone to calcify—far more so than any other normal tissue in the body—so that in old age more or less calcification and ossification always takes place in tracheal, costal, laryngeal, and articular cartilages. That is to say, the difference between the different cartilages in respect to calcification is one of degree only, which may not be discernible under the unnatural conditions of an experiment *in vitro.*

Furthermore, if we not only consider the cartilages, but also take into account the nature of the tissues in which pathological calcification takes place, we find further support for the physical theory. What have these many and various sites of calcification in common? Certainly not a common chemical composition, for we find most widely differing sorts of materials the seat of calcium deposition; contrast, for example, the elastic coat of arteries, ganglion cells, necrotic epithelium, and hyaline cartilage; in fact every dead or dying tissue may calcify. What these various tissues do have in common are: (1) a poor blood-supply and slow lymph-stream, with conditions retarding the rate of exchange by osmosis; (2) a more or less homogeneous, usually hyaline, ground substance.

Even in metastatic calcification a hyaline ground substance seems to be the point of election for the deposition of the calcium salts, for Huebschmann [70] found that in the lungs in this condition the deposit is first and chiefly in the elastic fibres. Furthermore, that tissues which are to calcify have a high affinity for inorganic salts is shown by their well-known tendency to lay on iron when this is available, *e.g.*, in the vicinity of hemorrhages.[71]

Taking ossification and calcification by and large as the processes occur, we cannot fail to be impressed with these two factors of a homogeneous ground substance and a poor circu-

[70] Huebschmann: Histologie der Kalkmetastase, Centralbl. d. allg. Path., 1908, xix, 737.

[71] S. Ehrlich (Eisen und Kalkimpragnation in menschlichen Geweben, Centralbl. f. allg. Path., 1906, xvii, 177) holds that the iron serves as a mordant for the calcium which is subsequently deposited.

lation; pathologists especially have been familiar with the coincidence and have repeatedly called attention to it (see Ricker,[72] Aschoff[73]). Therefore, all things considered, we are fairly driven back to the conception of a physical *Kalkfänger,* which serves as a point where condensation of the poorly soluble calcium salts takes place until, perhaps because of variation in CO_2 content in the solvent fluids, precipitation begins and continues rhythmically as Hofmeister has suggested. Precipitation is ordinarily in excess of re-solution because of the known slowness of solution of precipitates by a fluid in which the precipitated substance exists at all times nearly at the saturation point, although when for any length of time the amount of calcium in the blood is reduced resolution exceeds deposition, and absorption of calcium occurs.[74]

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[72] Ricker: Verkalkung und Steinbildung, Ergebn. d. allg. Path. (Lubarsch-Ostertag's), 1896, iii, 643.

[73] Aschoff: Verkalkung, Ergebn. d. allg. Pathol. (Lubarsch-Ostertag), 1902, viii, 561.

[74] Lichtwitz (Ueber die Bedeutung der Kolloide für die Konkrementbildung und die Verkalkung, Deutsch. med. Wochenschr., 1910, xxxvi, 704) has suggested another hypothesis of calcification which can be applied to pathological calcification, perhaps, but not so well to ossification. He considers especially the part played by the colloids in keeping the calcium salts in solution, and notes that in areas where calcification occurs we have had a precipitation of these colloids; *e.g.,* in caseous areas there has been precipitation of proteins, in atheroma the fatty changes represent a precipitation of lipoids. When in these areas the colloids are thus precipitated the calcium salts which they have kept in solution will then fall out. Of course the amount of dissolved calcium salts present in the area at any one time would not, when precipitated, cause any noticeable calcification, but because of the withdrawal of the calcium from the solution the osmotic equilibrium is disturbed, and there being now a relatively greater concentration of calcium salts in the surrounding fluids, these will continue to diffuse into this area and to be precipitated until the area is entirely filled up with precipitated calcium salts. This is an attractive hypothesis, but objections may be raised. In the first place, it does not seem possible to apply it to ossification, and in the second place it seems probable that under the conditions specified the invading calcium would be promptly precipitated on the inner surface, or even in the walls of the diffusion membrane, thus blocking further infiltration.

Taking all the evidence as it stands we find ourselves best satisfied with that which indicates that *calcification begins as a simple physical adsorption by hyaline substances,* which have a more or less specific adsorption affinity for calcium. That the substances which take up calcium have strong and specific adsorption affinities is well established. For example, cartilage itself is the richest of all tissues in NaCl (Bunge [52] found that 70.7 per cent. of the ash of nasal cartilage from the pig is NaCl), yet contains very little potassium, although the circulating blood contains much potassium, and in most tissues the proportion of potassium is greater than of sodium. In the shark, indeed, so great is the affinity of the cartilaginous skeleton for NaCl that this easily soluble salt takes almost the same place as does calcium in the mammals, constituting 16.69 per cent. of the total fresh weight and 94.24 per cent. of the ash of the cartilage (Petersen and Soxhelet [75]). On the other hand, in rickets the cartilage seems unable to take up even the calcium which is present in the blood and tissues in normal amounts (Brubacker [76]). As before mentioned, cartilage also shows a decided adsorption affinity for uric acid and many other crystalloids.

The hyaline degenerated tissues in which calcification commonly occurs also show a strong adsorption affinity, especially for pigments, such as iron, and often for various dyes that stain by physical rather than by chemical union. May we not, indeed, liken the process of calcification to its analogues, the infiltration of foreign bodies in the bladder with urinary salts, or even the petrifaction of trees and similar objects? Schade [50] points out that in the former case the part played by the foreign body is not to be compared to the starting of crystallization, since in crystallization the substance initiating crystallization must be of the same nature as the crystals, and the solution

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[75] Petersen and Soxhelet: Ueber die Zusammensetzung des Knorpels vom Haifisch, Jour. f. prakt. Chem., 1841, vii, 179.

[76] Brubacker: Ueber den Gehalt an anorganischen Stoffen, besonders an Kalk, in den Knochen und Organen normaler und rachitischer Kinder, Ztschr. f. Biol., 1890, xxvii, 517.

must be saturated. Rather is it to be considered as a surface phenomenon, concentration at the surface of colloidal particles leading to saturation and precipitation, which is progressive as long as new supplies come in, and permanent when the stroma of the precipitated mass is a non-reversible colloid. After all, to what extent does a mass of calcifying dead tissue in the body, *e.g.*, a thrombus, differ from the saturation of a fibrin clot in the urinary bladder with the salts of the urine? In each case the colloid is permeated by a fluid nearly saturated with certain salts, and when the process is completed we have alike in each a ground substance of irreversibly coagulated colloids in which the crystalloidal deposit is held. We do not question that the formation of the urinary concretion may be initiated by purely physical causes; then why not admit the same possibility for calcification in the tissues?

RECAPITULATION

So far as the knowledge gained from the literature and personal investigation permits me to form opinions, my present understanding of the essentials of calcification and ossification may be summarized as follows: Calcium is carried in the blood in amounts not far from the saturation point, held in solution by the colloids and the carbon dioxide, and existing probably in the form of an unstable double salt of calcium bicarbonate and di-calcium phosphate. In normal ossification, and in most instances of pathological calcification, the deposition is probably initiated by a process of colloidal adsorption causing a concentration of this double salt in the hyaline matrix which is to be calcified, and which has a strong affinity for calcium salts. Reduction in the amount of carbon dioxide in such areas, or some unknown agency, causes a precipitation of calcium salts in this colloid matrix, and permits of further infiltration of dissolved calcium salts whenever the concentration of CO_2 in the fluids may be greater (Hofmeister). The composition of bone and of most pathological deposits of calcium exhibits an almost constant ratio of phosphate (from 85 to 90 per cent.) and carbonate (from 10 to 15 per cent.), which constancy of composi-

tion is to be ascribed to the relative solubility of these calcium salts in the blood, and the approximately constant composition of this solvent. Slight variations in the composition of the blood may cause corresponding slight changes in the composition of the calcium deposits in the body. It is inconceivable that a mass of calcium salts anywhere in the body can for long possess a chemical composition essentially different from that of the bone, with which it is in constant exchange through the medium of the circulating blood.

There is no acceptable evidence that in ossification, or ordinarily in pathological calcification, the deposition of calcium is initiated as a chemical precipitation by some precipitating ion present in the tissues which are to be calcified. *From the beginning the calcium seems to be deposited as carbonate and phosphate in about the same ratio as in mature bone.*

Hyaline cartilage possesses an affinity for calcium which is not exhibited to an equal degree by other tissues, and this affinity is more marked in cartilage which normally ossifies than in cartilages which normally do not ossify. This specific affinity does not depend on any functional activity of the cells, for it is shown by dead cartilage. No difference in chemical composition can be found to explain this difference between ossifying and non-ossifying cartilage in regard to their absorption affinity for calcium salts. A homogeneous, hyaline structure is the usual characteristic of calcifying substances, which resemble each other much more in physical qualities than in chemical composition.

There seem to be no essential differences between the processes involved in normal ossification and in most instances of pathological calcification; any area of calcification may be changed to true bone in the course of time. Calcium salts seem to exert a specific influence on connective-tissue cells, causing them to form bone; without this stimulus they cannot form bone, at least not readily and normally.

Exceptional cases of calcification occur in which other processes are involved than in ossification. One of these, "metastatic calcification," occurs whenever from any cause the

proportion of calcium present in the blood is so great that it requires the effect of both the colloids and of the CO_2 in maximum concentration to keep it in solution; then the calcium salts are deposited in those points in the body where the CO_2 content of the fluids is least. Another exception is seen whenever there is a considerable splitting of fats, the new-formed fatty acids in some cases combining with calcium to form calcium soaps. These calcium soaps are ordinarily absorbed, but exceptionally, when in large amounts, *e.g.*, calcifying lipoma, etc., the fatty acid radicals may be replaced by P_2O_5 and CO_2. There is no satisfactory evidence, however, that this is a common, much less a usual method of calcification, and there is much evidence that it is not. *Calcium deposition seems to depend, alike in normal and in most pathological conditions, rather on physico-chemical processes than on chemical reactions.*